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BIOLOGICAL COUNTERMEASURES FOR THE CONTROL OF HAZARDOUS MATERIAL SPILLS

by

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Grant No. R 802207

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16. ABSTRACT

The possibility of accidental spills of hazardous substances poses a constant threat to the waters of the nation. Effective ways to control such spills and to mitigate their effects include physical and chemical techniques, but biological countermeasures have not been considered feasible to date. Determining the feasibility of this countermeasure was the primary focus of this study.

Using the hazardous compounds, phenol and methanol, as test substances, treatability studies were performed using acclimated bacteria to estimate their growth kinetic and substrate removal rates and the effects of those coefficients of environmental variables such as temperature, pH, and salinity in ranges found typically in fresh and estuarine waters. Numerical and graphical methods were developed to select the required amount of bacterial solids to remove some initial amount of phenol and methanol within a selected period of time in situations approximating a contained spill. The biological countermeasure's effectiveness was tested in simulated spill situations in lentic and lotic environment laboratory systems, and the deleterious effects of applying the countermeasure were examined through tests involving oxygen depletion and alterations in primary production.

Biological countermeasures were shown to be a feasible method for hazardous material spill removal within certain limitations imposed by the toxicity of the material to bacteria and its initial concentration.

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FOREWORD

The U.S. Environmental Protection Agency was created because of increasing public and government concern about the dangers of pollution to the health and welfare of the American people. Noxious air, foul water, and spoiled land are tragic testimonies to the deterioration of our natural environment. The complexity of that environment and the interplay of its components require a concentrated and integrated attack on the problem.

Research and development is that necessary first step in problem solution; it involves defining the problem, measuring its impact, and searching for solutions. The Municipal Environmental Research Laboratory develops new and improved technology and systems to prevent, treat, and manage wastewater and solid and hazardous waste pollutant discharges from municipal and community sources, to preserve and treat public drinking water supplies and to minimize the adverse economic, social, health, and aesthetic effects of pollution. This publication is one of the products of that research and provides a most vital communications link between the researcher and the user community.

A number of methods, including biological countermeasures, have been considered for the control of hazardous material spills. Biological degradation, while attractive in some respects, suffers from several difficulties: the necessity of having on hand large quantities of acclimated cultures; problems associated with stockpiling many such cultures, each of which is specific to a particular substance; and the apparent resistance of many hazardous materials to biological degradation. This report summarizes an investigation on the feasibility of using microbiological processes to mitigate hazarous material spills in watercourses and should be of interest to all those concerned with building up an arsenal of countermeasures for hazardous material incidents.

Francis T. Mayo, Director
Municipal Environmental Research Laboratory

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ABSTRACT

The possibility of accidental spills of hazardous substances poses a constant threat to the waters of the nation. Effective ways to control such spills and to mitigate their effects include physical and chemical techniques, but biological countermeasures have not been considered feasible to date. Determining the feasibility of this countermeasure was the primary focus of this study.

Using the hazardous compounds, phenol and methanol, as test substances, treatability studies were performed using acclimated bacteria to estimate their growth kinetic and substrate removal rates and the effects of those coefficients of environmental variables such as temperature, pH, and salinity in ranges found typically in fresh and estuarine waters. Numerical and graphical methods were developed to select the required amount of bacterial solids to remove some intial amount of phenol and methanol within a selected period of time in situations approximating a contained spill. The biological countermeasure's effectiveness was tested in simulated spill situations in lentic and lotic environment laboratory systems, and the deleterious effects of applying the countermeasure were examined through tests involving oxygen depeltion and alterations in primary production.

Biological countermeasures were shown to be a feasible method for hazardous material spill removal within certain limitations imposed by the toxicity of the material to bacteria and its initial concentration.

This report was submitted in partial fulfillment of Grant No. R802207 by the University of Texas at Austin under the sponsorship of the U.S. Environmental Protection Agency. This report covers the period March, 1973 to March, 1975.

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ABBREVIATIONS AND SYMBOLS

a --Cell yield coefficient (mg/l VSS produced)/(mg/l TOC utilized)
a' --Oxygen required per substrate utilized (mg/l O_2 required)/(mg/l TOC utilized)
A_a --Air-water interface area (L^2)
A_c --Surface area of cloth bag (L^2)
A_r --Cross-sectional area of river (L^2)
A_s --Surface area of water body at quiescent condition (L^2)
b' --Oxygen consumption rate for endogeneous respiration (1/T), (mg/l O_2 required)/(mg/l VSS)/hr
C --Material exchange coefficient (L/T), cm/hr
c,c_1,c_2 --constants
D --Dispersion coefficient for dissolved pollutants (L^2/T)
D' --Dispersion coefficient for cloth bags (L^2/T)
D_a --Average diameter of air bubble
E --Cloth bag efficiency
e --Surface area expansion coefficient caused by turbulence
f --Correction factor for the substrate removal rate coefficient, k
f_{c_o} --Cloth bag distribution function in river (1/L)
ΔF_{ox} --Free energy of oxidation at standard condition, Kcal/mole
G --Mean temporal velocity gradient (1/T), sec^{-1}
g --gravity constant (L/T^2)
H --Hydraulic mean depth of river (L), or reactor depth (L)ft,
h_f --Head loss (L)
H_{min} --Reactor depth that provides the minimum stripping rate
I --Energy gradient
k --Substrate removal rate coefficient for biological decomposition (1/T), hr^{-1}
k_T --k at temperature T°C (1/T), hr^{-1}
k_a --reaeration rate coefficient (1/T), hr^{-1} or day^{-1}
k_d --Cell decay coefficient (1/T), hr^{-1}
k_e --Stripping rate coefficient (1/T), hr^{-1}
k_r --Substrate removal rate coefficient contributed by other than biological decomposition (1/T), hr^{-1}
K_s --Michaelis-Menten constant (M/L^3), mg/l as TOC
K_v --Volatilization rate coefficient (L/T), m/hr
L --Likelihood function
M_s --Total mass of substrate (M)
M_{so} --Total mass of substrate initially spilled (M)
M_x --Total biomass applied (M), as VSS
N --Number of observations or number of cloth bags applied
n --Coefficient of roughness
O --Dissolved oxygen concentration (M/L^3), mg/l
O_s --Saturated dissolved oxygen concentration (M/L^3), mg/l
Pr_b --Sludge production rate in batch treatment systems (M/T), M as VSS
Pr_c --Sludge production rate in CSTR systems (M/T), M as Vss
Q --Flow rate (L^3/T)
Q_a --Stripping air flow rate (L^3/T)

Q_w --Waste sludge flow rate (L^3/T)
R --Hydraulic radius (L), m
r --Relative efficiency of CSTR system
R_1 --Residual in the multinomial regression for the evaluation of bacterial growth kinetics
R_2 --Residual in the multinomial regression for the evaluation of substrate removal kinetics
Rr --Oxygen utilization rate (M/L^3T), (mg/l O_2)hr
Rr_1 --Observed Rr
Rr_2 --Theoretically computed Rr
S --Substrate concentration (M/L^3), mg/l as TOC
S_c --Substrate concentration inside a cloth bag (M/L^3), mg/l as TOC
S_e --Effluent substrate concentration (M/L^3), mg/l as TOC
S_o --Influent substrate concentration or substrate concentration at time t_o (M/L^3), mg/l as TOC
S_s --Saturated volatile substance concentration for a given partial vapor pressure (M/L^3)
T --Temperature, °C
t --Time (T)
t_a --Sludge application time measured from the pollutant spill (T)
t_d --Detention time in a stretch of a river or detention time of air bubble in a reactor (T)
t_o --Beginning time of a sampling interval (T)
u --Mean velocity of river (L/T)
V --CSTR or batch reactor volume (L^3)
V_b --Buoyancy velocity of air bubble (L/T)
V_c --Cloth bag volume (L^3)
X --Biomass concentration (M/L^3), mg/l as VSS
X_c --Biomass concentration inside a cloth bag (M/L^3), mg/l as VSS
θ --Temperature coefficient for substrate removal rate coefficient
μ --Absolute viscosity of water (M/LT)
ρ --Density of water (M/L^3)
σ --Standard deviation of X (dimension of X)
τ_b --Aeration time required to achieve an effluent quality S_e in a batch reactor (T)
τ_c --Hydraulic detention time in CSTR to achieve an effluent quality S_e(T)

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SECTION 1

INTRODUCTION

NEED FOR STUDY

The possibility of accidental spills of an ever increasing volume and array of hazardous substances produced and shipped by industry poses a constant threat to the waters of the nation. These spills cause varying degree of hazard and damage to a watercourse, depending on the nature and uses of the watercourse and the type and quantity of material spilled. The Federal Water Pollution Control Act of 1972 declared that the policy of the United States was to prohibit the discharge of hazardous substances into navigable waters of the United States, and in Section 311(c)(2) of the Act provisions were made for the preparation of a national contingency plan for the removal of oil and hazardous substances. This plan, published August 13, 1973 in the Federal Register as the National Oil and Hazardous Substances Pollution Contingency Plan (40 CFR 1510), delineates the actions that may be taken to respond to a spill of hazardous materials. These actions are: Phase I - Discovery and Notification, Phase II - Evaluation and Initiation of Action, Phase III - Containment and Countermeasures, Phase IV - Removal, Mitigation, and Disposal, and Phase V - Documentation and Cost Recovery.

Containment and removal or mitigation of the spilled hazardous substance are part of Phases III and IV. In Phase III, Containment and Countermeasures, defensive actions are to be initiated as soon as possible after discovery and notification of a discharge. These actions may include, among other things, the placement of physical barriers to halt or slow the spread of the pollutant and its effects on water-related resources. In Phase IV, Cleanup, Mitigation, and Disposal, actions are taken to recover the pollutant from the water and affected public and private shoreline areas, and monitoring activities are initiated to determine the scope and effectiveness of removal actions. Actions that may be taken include: (1) the use of sorbers, skimmers, and other collection devices for floating pollutants, (2) the use of vacuum dredges or other devices for sucking pollutants, (3) the use of reaeration or other methods to minimize or mitigate damage resulting from dissolved, suspended, or emulsified pollutants, or (4) special treatment techniques to protect water supplies and wildlife resources from continuing damage (including biological countermeasures).

A number of methods, including biological countermeasures, has been considered for the control of hazardous material spills. These methods have been reviewed by Dawson _et_ _al_. (1972), who concluded that:

> "biological degradation, while attractive in some respects, suffers from several difficulties. In order for degradation to proceed at a rapid rate, it would be necessary to have on hand large quantities of acclimated cultures. The problems associated with stockpiling many such cultures, each of

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> which is specific to a particular substance, are obvious. Also, many hazardous materials are apparently resistant to biological degradation."

While this evaluation points out the difficulties of biological countermeasures, it does not rule out their use. The extent of the difficulties may not be as great as first thought and the possibility of overcoming them has not been specifically investigated with regard to spilled hazardous substances.

A large body of literature and experience exists in the waste treatment field that could contribute to a further evaluation of biological countermeasures. For example, most or all of the hazardous materials shipped and spilled are products of some manufacturing process that produces a waste residual. This waste may contain varying amounts of the chemical manufactured and is usually treated before being discharged to the environment to remove the hazardous substances from the waste stream or to reduce their concentration to the extent that they would no longer be considered hazardous. As a result, waste treatment technology offers possible solutions to the control of hazardous material spills. In particular, the use of microorganisms in biological waste treatment has been developed to a sophisticated state and has been applied to most types of industrial wastes. Biological waste treatment has shown to be highly effective in removing a large number of hazardous substances from waste streams as long as these toxic substances are used by the microorganisms as a food substrate (Dawson et al., 1972). In addition, it is often possible to acclimate bacteria to seemingly nonbiodegradable materials. The feasibility of biodegradation has also been demonstrated by recent reports (Chears and Myers 1973; and A.P.I., EPA, and U.S.C.G 1973) that indicate that bacteria may be viable countermeasures for the mitigation of oil spills.

Bacterial cultures may be stored for long periods of time in a dormant state and then later constituted for use. In the dormant state, the bacteria are in a frozen or powder form, amenable to storage and to rapid, easy deployment without highly specialized equipment.

Because the use of microorganisms as a countermeasure for hazardous material spills appears to fit the criteria for potential countermeasures suggested by Dawson et al., (1972) and because little information is available to adequately assess the feasibility of using microorganisms as a biological countermeasure, the need for such a study became evident.

OBJECTIVES OF STUDY

This investigation, entitled "Biological Countermeasures to Mitigate the Effects of Hazardous Material Spills," was initiated and funded by the Environmental Protection Agency (Grant #R802207). The overall objective of the study was to investigate the feasibility of using microbiological processes to mitigate hazardous material spills in watercourses. Several more specific objectives were defined as follows:

1. Investigate the response requirements for any hazardous material spill and determine the response requirements using microorganisms as a biological countermeasure.

2. Conduct screening tests to determine candidate microorganisms for mitigating the effects of certain of the Environmental Protection Agency's priority-ranked soluble hazardous polluting substances.

3. Conduct small-scale ecological system studies to assess total ecosystem response to these hazardous materials and their decomposition products.

4. Select candidate microorganisms for the priority list of hazardous materials and conduct simulated spill experiments on a laboratory scale, deploying these microorganisms.

5. Develop production, storage, reculture, and deployment methods for the microorganisms selected.

6. Evaluate the practical feasibility of biological countermeasures for mitigation of hazardous material spills.

It was the intent of this work to emphasize the evaluation of the feasibility of using microorganisms as a countermeasure to mitigate the effects of hazardous material spills in the environment. Development of data on: (1) growth requirements and environmental factors affecting growth of microorganisms found to successfully break down the hazardous materials, (2) the fate of these materials and their byproducts in ecological systems, and (3) small spills into small ecological systems was considered necessary for evaluation of the feasibility of using biological countermeasures for mitigating effects of hazardous materials.

SCOPE OF STUDY

To accomplish the stated objectives, it was necessary to conduct literature surveys to determine the experience of other investigators in using microorganisms to break down hazardous materials and to determine the information available on the effects of these hazardous materials in ecological systems.

The experimental work included laboratory culture of microorganisms, starting with enrichment cultures to assess the types of microorganisms available to break down various hazardous materials, followed by the determination of growth rate characteristics of these microorganisms, including effects of environmental factors. Other laboratory tests were conducted using small, contained, aquatic ecological systems to determine the fate of the selected hazardous materials and of their breakdown products in the water, sediment, and biota of natural systems. Finally, laboratory tests were also conducted on simulated spills of these hazardous materials in contained, aquatic ecological systems. These systems were large enough to represent portions of several types of environmental systems so that the results could be realistically applicable to natural systems, and the feasibility of using microorganisms as a countermeasure could be rationally assessed.

Investigations were made into existing programs for responding to spills of hazardous materials, the response requirements of such programs, and the requirements of such programs were they to use biological countermeasures instead of chemical or other measures. In the latter stages of this project an evaluation was made of the tactical feasibility of using microorganisms as a countermeasure for the spill of hazardous materials.

SECTION 2

CONCLUSIONS

The conclusions of this study are as follows:

1. Based on criteria for assessing potential countermeasures for mitigating hazardous material spills, biological countermeasures appear useful because:

   a. Microorganisms are highly effective in removing certain hazardous materials.

   b. Microorganisms that attack a variety of hazardous materials exist. (e.g., the Pseudomonads).

   c. It should be possible to easily and rapidly deploy microorganisms *in situ* or in a portable treatment system in a fresh liquid state, a powdered state, or a freshly reconstituted state.

   d. Potentially harmful secondary effects should be minor because microorganisms are a natural part of the aquatic environment, pathogenic bacteria will not likely constitute a significant part, if any, of the countermeasure, noxious sludge should not be formed, and microorganisms should not persist since they should metabolize their own protoplasm following consumption of the hazardous material and disappearance of the food source.

2. Previous investigations of some of the most well-known hazardous materials show that the majority are biodegradable.

3. Based on treatability tests for phenol, methanol, and nitrophenol:

   a. The following kinetic equations:

$$\frac{dX}{dt} = -a\frac{dS}{dt} - k_d X \text{ and } \frac{dS}{dt} = -\frac{kXS}{K_s + S},$$

   satisfactorily described the bacterial growth and substrate removal kinetics using phenol and methanol, where X is biomass concentration (mg/l), S is substrate concentration (mg/l), t is time, a is cell yield coefficient (biomass produced/substrate utilized), k_d is cell decay coefficient (time^{-1}), K_s is the Michaelis-Menten constant (mg/l), and k is substrate removal rate coefficient (time^{-1}).

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b. The cell yield coefficient, a, and the Michaelis-Menten constant, K_s, changed insignificantly with temperature.

c. The Michaelis-Menten constant, K_s, was estimated to be 236 mg/l with a standard deviation of 70 mg/l for phenol and 2,330 mg/l with a standard deviation of 1,410 mg/l for methanol, based on total organic carbon (TOC).

d. The cell yield coefficient, a, was estimated to be 1.21 with a standard deviation of 0.06 for phenol and 1.25 with a standard deviation of 0.45 for methanol, based on TOC (mg/l) and volatile suspended solids (VSS)(mg/l).

e. The endogeneous respiration rate was closely related to the substrate utilization rate coefficient. Thus, $k_d = 0.066\ K^{0.87}$ and $k_d = 0.0115\ k^{0.634}$ (k and k_d are based on the unit of hr^{-1}) are proposed for the prediction of cell decay coefficients for phenol- and methanol-acclimated activated sludges, respectively.

f. The oxygen utilization rate can be formulated as $Rr = -a'(ds/dt) + b'X$, where Rr is the oxygen utilization rate in mg/l O_2/hr, a' is a coefficient designating oxygen requirement per substrate utilized, b' is a coefficient designating oxygen requirement per biomass for endogenous respiration, S is substrate concentration, X is biomass concentration, and t is time. Based on substrate concentration as TOC (mg/l) and biomass concentration as volatile suspended solids (mg/l), the values a' = 1.39 for phenol, a' = 2.23 for methanol, and $b' = 1.42\ k_d$ (b' and k_d have the units hr^{-1}) are proposed.

g. An initial lag phase was observed in 8 out of 115 phenol batch tests and in 31 out of 66 methanol batch tests. The average duration of the initial lag phase among the observed cases was 8 hours for phenol and 9 hours for methanol, and the average aeration lag time due to the lag phase was 5 hours for phenol and 8 hours for methanol.

h. The modified Arrhenius equation, $k_{T2} = k_{T1}\theta^{(T2-T1)}$ did not describe the temperature effect on k properly because the temperature coefficient, θ, changed with temperature range. However, θ ranged between 1.0 to 1.4 depending on substance (phenol or methanol), pH, salinity, and temperature.

i. The primary factor affecting the substrate decomposition rate in natural systems is pH. Phenol decomposition results in a considerable decrease in pH so that the buffering capacity of the water is the most important factor. Methanol decomposition does not affect pH significantly, thus the initial pH of the water is the most important factor.

j. In batch treatment of 770 mg/l of phenol and 1,000 mg/l of methanol (as TOC), nitrogen and phosphorus did not have any recognizable effect on k, while trace elements, such as Fe^{++}, Mg^{++}, Mn^{++}, Ca^{++}, and Zn^{++}, showed a slightly perceptible effect.

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k. The absence of extra-cellular nitrogen and phosphorus resulted in a greater cell yield coefficient, a, in phenol tests. However, the cells grown in this condition were found to decay more rapidly than normal cells.

4. Based on the treatability tests, the following conclusions are made pertaining to the use of batch treatment systems as a countermeasure:

a. Batch treatment systems are preferred over continuous-stirred tank-reactor (CSTR) systems for spills of phenol and methanol, especially when the spill concentrations are high. Batch systems require much less aeration time to achieve a certain effluent quality and produce more acclimated sludge than CSTR systems.

b. Batch systems can be designed using numerical methods or using batch kinetic diagrams.

c. Sludge-containing cloth bags were found useful for easy containment of the sludge when consecutive batch treatments were required. Floating cloth bags can be used for *in situ* treatment methods to prevent sludge settling.

d. The material transport rate through sludge-clogged, cloth bags can be expressed as:

$$V_c \frac{dS_c}{dt} = CA_c(S-S_c),$$

where V_c is the volume of cloth bag, S_c is the substance concentration inside the cloth bag, S is the concentration outside the bag, t is time, C is the material exchange coefficient (L/T), and A_c is the surface area of the bag.

e. Between methanol concentrations of 4,000 mg/l and 6,000 mg/l (VSS), the material exchange coefficient, C, remained the same. In the turbulent flow regime, C showed a linear relationship with velocity. The value of C for a phenol sludge concentration of 25,000 mg/l (VSS) was approximately one-half of that for a methanol sludge.

f. For phenol sludge (15,000 mg/l to 30,000 mg/l as VSS), $C = 0.0710$ cm/hr in a laminar flow regime and $C = 0.159\ G^{2/3}$cm/hr in a turbulent flow regime. For methanol sludge (4,000 mg/l to 6,000 mg/l as VSS), $c = 0.141$ cm/hr in a laminar flow regime and $C = 0.317\ G^{2/3}$ cm/hr in a turbulent flow regime. These values or equations are proposed for the prediction of C where G is the mean temporal velocity gradient (sec^{-1}).

g. When sludge-containing cloth bags are used for the removal of spills, the substrate removal rate by organisms can be expressed as:

$$\frac{ds}{dt} = - \frac{EkXS}{K_s+S}$$

where E is the cloth bag efficiency, which is obtained from cloth bag efficiency diagrams.

h. When sludge-containing cloth bags are used in a batch treatment system the system can be designed in the same manner as a regular batch system except that Ek instead of k is used for the substrate removal rate coefficient. The aeration time required to achieve a given removal was observed to be slightly less than the theoretically computed time, probably owing to organisms that escaped from the cloth bags.

5. Based on spill control tests in a model river, the following conclusions can be made:

a. Application of bulk sludge in streams is not an efficient method for phenol and methanol removal because of sludge settling. Floating cloth bags may be used to prevent this problem; however, this method is highly restricted by the reaeration capacity of streams and the large amount of acclimated sludge required.

b. Fixed, confining barriers may be used to prevent the dispersion of spills. Once pollutants are contained within barriers they may be treated in a batch manner. Cloth bags may be employed when the mixing intensity is not sufficient for complete suspension of sludge. Oxygen need only be supplied within or near the cloth bags.

6. Based on model lake tests, the following conclusions can be made:

a. Phenol spills contained by a barrier can be removed using unacclimated sludge from a local activated sludge domestic waste treatment plant.

b. Use of biological countermeasures will result in a significant impact on the dissolved oxygen resources in the aquatic system. However, this impact can be reduced by mechanical aeration.

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SECTION 3

RECOMMENDATIONS

It is recommended that development of biological countermeasures should continue. Major research needs include:

1. Studies on countermeasure storage and reconstitution to determine the shelf-life of the stored material, the need for additions of mineral salts, and the amount of material needed for spills of a given chemical.

2. Development of techniques for countermeasure application in quiescent and flowing systems.

3. Determination of additional candidate chemicals for application of biological countermeasures.

4. Further confirmation of the methods described in this report for calculating amounts of the countermeasure needed for a given volume spill.

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SECTION 4

DEVELOPMENT OF INFORMATION FOR BIOLOGICAL COUNTERMEASURE FEASIBILITY DETERMINATION

ELEMENTS OF SPILL CONTROL

The National Oil and Hazardous Substances Pollution Contingency Plan (40 CFR 1510) delineates five classes of actions that comprise the elements of spill control. These actions are: Phase I - Discovery and Notification (discovery of a spill by the discharger, patrol vessels, or incidental observation and the reporting of that discovery to the proper agency), Phase II - Evaluation and Initiation of Action (evaluation of the magnitude and severity of the spill, the feasibility of removing it, and the effectiveness of removal actions), Phase III - Containment and Countermeasures (actions taken to restrain the movement of the spilled material and to minimize its effects on water-related resources), Phase IV - Cleanup, Mitigation, and Disposal (actions taken to recover the spilled material and to monitor the scope and effectiveness of removal actions), and Phase V - Documentation and Cost Recovery. The time needed to implement any of these phases will depend on the location of the spill, the material spilled, the magnitude of the spill, and so forth. Employment of a biological countermeasure imposes special constraints on the activities in Phases III and IV and requires that its use be carefully considered in Phase II. To understand these special constraints, the requirements of a general countermeasure and the information needed to judge the suitability of biological countermeasures will be discussed in this section.

REQUIREMENTS OF COUNTERMEASURE

Dawson _et_ _al_., (1972) suggested the following criteria for evaluating potential countermeasures:

1. Countermeasures should be highly effective.

2. Countermeasures should be applicable to a large number of substances.

3. Countermeasures should be amenable to rapid, easy deployment. (Highly specialized equipment and/or chemicals that require extensive stockpiling prior to a pollution incident or that cannot be rapidly conveyed to the scene of an accident are undesirable.)

4. Countermeasures should be free from potentially harmful secondary effects in the aquatic environment, including production of noxious sludges.

5. Countermeasures developed to combat spills of hazardous polluting substances should take advantage of available technology, particularly that developed to combat oil spills.

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Several physical and chemical countermeasures were evaluated and the difficulties of biological countermeasures were discussed. The authors also discussed the dynamics of a spill and the problems of containment and mitigation given the type of material spilled and the nature of the receiving water. The most important parameter, they concluded, was the time lag between the spill and the initiation of treatment because effects on organisms and, in many cases, process removal efficiencies, are functions of the spilled material concentration. One could add to this response time the time required for removal of the spilled material to safe levels. Huibregtse et al., (1976) incorporated such requirements into a user's manual for hazardous material spills but did not include biological countermeasures.

For a biological countermeasure to be considered feasible, the first four criteria should be satisfied to the greatest extent possible and the biological countermeasure should be competitive with, or at least complimentary to, the physical/chemical countermeasures available. The experimental program developed in this project had as its primary focus the test of feasibility of biological countermeasures using the above criteria. The information needed to demonstrate such feasibility is discussed below.

INFORMATION NEEDED

The following information was considered essential to assess the feasibility of the biological countermeasure: (1) screening tests to determine the general effectiveness and applicability of the countermeasure to hazardous materials, (2) growth kinetics tests to determine removal rates, growth rates, application rates, etc. so that the requirements and logistics of countermeasure deployment could be determined, and (3) simulated spills to demonstrate (under near field conditions) the feasibility and effectiveness of the countermeasure. Each of these items is discussed more fully below.

Screening Tests

Screening tests are simplified versions of growth kinetics tests and are conducted as a short-term, batch test. The purpose is to show in a short time, with little effort, whether the hazardous material being tested is biodegradable and, if so, identify the organisms that are involved In this study, screening tests were performed after the literature survey and were used primarily to determine the candidate hazardous materials for further testing in the growth kinetics and simulated spills experiments.

Growth Kinetics Tests

The attractiveness of biological countermeasures for hazardous material spills is twofold: (a) bacteria are natural components of ecological systems and their use as a countermeasure will not constitute the introduction of a "foreign" material and (b) bacteria will metabolize organic hazardous materials to the principal end products carbon dioxide and water, according to the general equation:

$$C_XH_YO_Z + 0_2 \xrightarrow{\text{enzyme}} C0_2 + H_2O \qquad (1)$$

The microbial utilization of a hazardous material in a finite volume, mixed reactor is described by the following equation (Pearson, 1968):

$$\frac{dS_1}{dt}V = QS_0 - QS_1 - qX_aV, \tag{2}$$

where:

$\frac{dS_1}{dt}V$ = change in hazardous material mass in system

QS_0 = influent hazardous material mass

QS_1 = effluent hazardous material mass

qX_aV = hazardous material mass removed by cells

and:

S_0 = influent hazardous material concentration (mg/l),

S_1 = effluent hazardous material concentration (mg/l)

Q = flow into and out of the reactor (l/d),

X_a = average microorganism concentration in reactor (mg/l),

V = reactor volume (l),

t = time, and

q = hazardous material removal rate =

$$\frac{\text{mg hazardous material removed/day}}{\text{mg microbes}}$$

For a system with no flow (e.g., a batch reactor), Equation 2 reduces to:

$$\frac{dS}{dt} = -qX_a, \tag{3}$$

which has the solution:

$$S = -qX_at, \tag{4}$$

where:

$$q = \frac{S_0 - S}{X_at}\ . \tag{5}$$

Eckenfelder (1970) has described this same process by the equation:

$$\frac{dS}{dt} = -kX_aS, \tag{6}$$

where:

S = hazardous material concentration (mg/l),

X_a = average microorganism cell concentration in reactor (mg/l), and

k = removal rate (mg S remaining/day/mg S/mg X_a).

In Equation 6, the product kS is equivalent to the term q in Equation 3 and in fact, Eckenfelder (1970) found that the solution to Equation 4 may be expressed as:

$$\frac{S_0-S}{X_a t} = kS. \tag{7}$$

These equations represent the overall biodegradation process, however, there are usually a number of biochemical reactions that take place in the microorganism as the hazardous material is reduced to elemental forms. This series of reactions may be referred to as the breakdown pathway.

The growth of bacteria in a reactor may be expressed by the following equation (Pearson, 1968):

$$\frac{dX_a}{dt} V = QX_0 - QX_1 + \mu X_a V - k_d X_a V, \tag{8}$$

change in cell mass = in - out + growth - decay in system

where:

X_0 = influent microorganism cell mass concentration (mg/l),

X_1 = effluent cell mass concentration (mg/l),

Q = flow into and out of system (l/d),

X_a = average cell mass concentration in system (mg/l),

μ = microorganism growth rate (mg cells produced/day/mg cells),

k_d = microorganism death rate (mg cells removed by death/day/mg cells),

t = time, and

V = volume.

For a system with no flow, Equation 8 reduces to:

$$\frac{dX_a}{dt} = (\mu - k_d)\, X_a. \tag{9}$$

It is important to note the nature of the relationship between the hazardous material concentration and bacterial growth rate. This relationship has been shown (Pearson,

1968) to be very similar to the Michaelis-Menton kinetic model for enzymatic action and may be expressed as:

$$\mu = \hat{\mu}\left(\frac{S}{K_s+S}\right), \tag{10}$$

where:

$\hat{\mu}$ = maximum growth rate (mg cells produced/day/mg cells),
S = hazardous material (haz. mat.) concentration (mg/1), and
K_s = hazardous material concentration at one-half the maximum growth rate (mg/1).

The techniques for deriving the maximum growth rate and the Michaelis Menton constant, K_S, have been given by Pearson (1968).

The substrate removal rate, q, may be transformed to the growth rate, μ, by multiplying by the yield coefficient, Y, as follows:

$$QY = \mu = \frac{\hat{\mu}\, S}{(K_s+S)} \tag{11}$$

$$\frac{\text{mg hazardous material remaining}}{\text{mg cells - day}} \times \frac{\text{mg cells produced}}{\text{mg hazardous material remaining}} = \frac{\text{mg cells produced}}{\text{mg cells - day}}$$

thus:

$$q = \frac{\hat{\mu}\, S}{Y\,(K_s+S)}\ . \tag{12}$$

Under steady state conditions, it may be shown from Equation 2 that q may be determined in a continuously stirred reactor by:

$$q = \frac{Q(S_0-S_1)}{VX_a}\ . \tag{13}$$

In a batch reactor, q may be calculated from Equation 5.

It is well known in microbiological research that the introduction of a small inoculum of bacteria into a medium with useable substrate initially results in growth of the bacteria at a maximum rate, $\hat{\mu}$, with concurrent reduction of the substrate concentration (see Figure 1) according to Equation 3. This period is termed the maximum growth phase. After a short time, the substrate concentration is reduced to a level that becomes limiting to the growth of bacteria and the bacterial concentration quickly reaches a maximum. This period is termed the declining growth phase. Following the peak, a decline in concentration occurs due to auto-oxidation and death; this is the death phase, or the often-called endogenous respiration phase.

Initially a delay in growth, called the lag phase, may occur. The extent of the lag phase is a function of the physiological condition of the bacteria, the size of the inoculum, the state of acclimation of the bacteria to the substrate, and perhaps other effects. Once growth begins, the maximum growth rate is characteristic of the bacteria, the other nutrients required by the bacteria for growth, the temperature of the medium, and the toxicity of the substrate. If the lag phase is very long, the rate at which the substrate is consumed also lags. Since one of the most important requirements of a countermeasure (Dawson *et al.*, 1972) is that it be capable of immediate use and application, the lag time must be minimized and the bacterial and substrate characteristics that influence the lag time must be defined.

Equations 3 and 9 may be used to describe the mitigation of a hazardous material spill and the increase in bacterial concentration, respectively, in a batch system or in a spill situation in which the spill occurred instantaneously (or over a very short time) and onto which bacteria were deployed. The reduction of the hazardous material and the growth of the bacteria would approximately follow the curve shown in Figure 1.

Equations 2 and 8, would be applicable to a continuous-flow biological treatment system that is operated such that high removal of the hazardous material and high bacterial retention in the system are achieved. Operational parameters for these treatment systems have been developed in practice for wastes containing hazardous substances (Eckenfelder, 1970). Equations may also be developed to describe the transport of hazardous materials in flowing systems, including biodegradation as well as other sink terms.

In order to apply these equations, their terms must first be obtained by experimental means. The constant for the growth rate-substrate relationship are especially important, as is the substrate removal rate determination.

Simulated Spills

Biological countermeasures may be employed in one of two ways: they may be applied *in situ* to a spill of a hazardous material, that is, in the receiving water itself, or they may be employed by pumping the spilled hazardous material to a portable biological treatment system brought to the site. For both treatment techniques, the nature and amount of the hazardous material spilled should be determined so that appropriate bacteria may be used and the proper amount of baterial culture applied. Containment of the spilled hazardous material is also desirable in order to avoid diluting effects of the natural system and to provide a controlled environment for the bacteria. Once the nature and amount of the hazardous material spilled have been determined and once containment has been achieved or at least ambient concentrations determined, the bacterial countermeasures may then be deployed. The amounts of countermeasure to be used, the time required for action, and the secondary effects must be determined experimentally.

The principal constraints of the biological countermeasure approach emerge in the production, storage, and deployment system and in the introduction of undesireable bacteria to a natural system. Production of a

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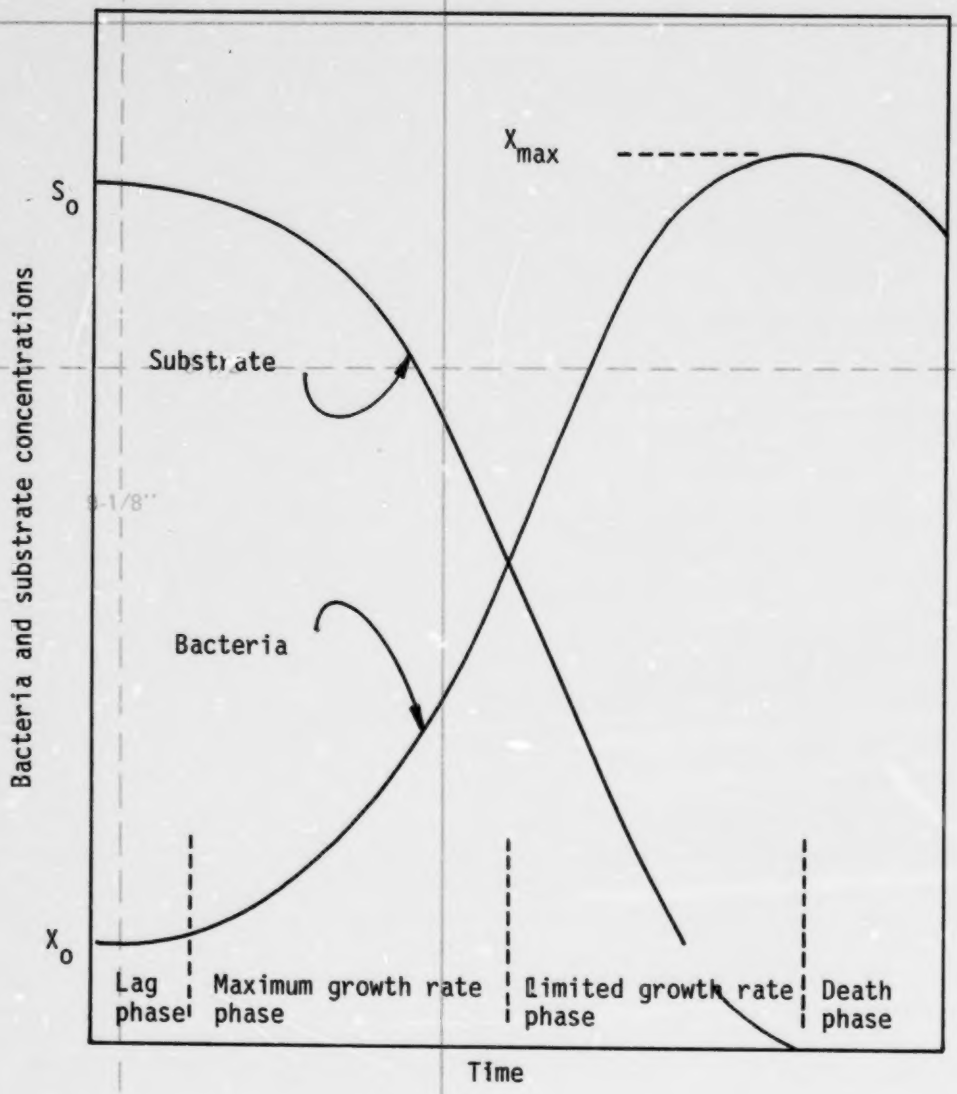

Figure 1. Microorganism - substrate relationship

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single bacterial species in large quantities (for use on a specific hazardous material) would impose a serious constraint on the feasibility of biological countermeasures. Thus, it is desirable to find bacteria that break down a wide variety of organic substrates and that are produced easily in large volume, such as the Pseudomonads. *Pseudomonas fluorescens* for example, may be grown on sugars, amino acids, organic acids, alcohols, aromatic compounds, and other cyclic organic compounds (Stanier 1950). Several species of the genus *Pseudomonas* other than *P. fluorescens* have been shown to use a variety of aromatic compounds (Gibson 1972, Chapman 1972).

Large quantities of bacterial material may potentially be stored in several forms: (1) as a liquid culture in which substrate is supplied continually and bacteria are produced continually, (2) in a frozen form in which large quantities of bacterial culture must be continually refrigerated, or (3) in a lyophilized, powdered form in which the bacteria are stored in large quantities in airtight containers. Storage in the liquid form is desirable in the sense that the bacteria are ready for immediate application, but the expense of maintaining these cultures at a number of points around the country near potential spill areas may be prohibitive. Storage in the frozen form is more desirable because less volume is needed, but continual refrigeration is required and a lag time is needed to reconstitute the bacteria for application. The lyophilized form may be the most desirable in terms of storage because the bacteria are in a powdered form, may be stored at room temperature, and may be maintained at many points around the country or even shipped with the hazardous material for which bacteria have been cultured. One possible disadvantage of this storage form is the time required (a few hours) for reconstitution of the bacteria to an active state. Because the storage method must be determined for each culture, investigation of this phase of deployment is required.

The methods for *in situ* deployment of the bacteria would depend on the physical/chemical state of the hazardous material in the receiving water and the storage mode of the bacterial culture. Spraying from a helicopter, boat, or from shore should be adequate. Physical deployent of the portable treatment plant and start-up of the bacterial culture appear to be the critical steps in the use of a biological waste treatment countermeasure.

Use of biological countermeasures may result in the addition of undesirable bacteria to a aquatic system. Application of pathogenic bacteria, for example, to a spill of a hazardous material could result in the proliferation of these bacteria as long as the hazardous material remains. Use of activated sludge from treatment plants for *in-situ* treatment or for the portable treatment plant may also result in the application of undesirable bacteria. However,this potential hazard may not be serious because the bacterial groups that break down hazardous materials will not likely be pathogenic and disinfection of the effluent from the portable treatment system should remove any undesirable bacteria from the waste stream before discharge to the aquatic system.

An undesirable effect of biological countermeasures in the consumption of dissolved oxygen by the bacteria during the breakdown of the hazardous material. Low levels of dissolved oxygen are typically found downstream of domestic and industrial waste discharges. This problem may be avoided by artificially aerating the receiving water after applying the bacteria or by using small amounts of bacteria such that excessive oxygen consumption does not occur.

Cautions about biological countermeasures are expressed in Annex X of the National Oil and Hazardous Substances Pollution Contingency Plan. The Plan states that biological countermeasures:

> "may be used only when such use is the most desirable technique for removing oils or hazardous substances and only after obtaining approval from the appropriate state and local public health and water pollution control officials. Biological agents may be used only when a listing of organisms or other ingredients contained in the agent is provided to EPA in sufficient time for review before its use."

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SECTION 5

SELECTION OF TEST MATERIALS

At the start of this project, 20 hazardous materials (individual compounds or groups of compounds) were selected for testing. By the end of the project, two compounds--phenol and methanol--had received most of the attention of the growth kinetics and spill simulation tests. The process by which the initial and subsequent selection of test compounds was accomplished is described in this section.

INITIAL SELECTION

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Contract List

In conjunction with the Project Officers for the Environmental Protection Agency, 14 compounds were selected from a list of 20 hazardous materials (Dawson et al., 1970). Of the 14 selected (Table 1), four were given a low experimental priority. From the physical/chemical characteristics shown in Table 2, it is apparent that several compounds are quite soluble in water and would be dispersed by natural mixing processes upon spillage. Several, like xylene and benzene, are relatively insoluble and would tend to float. Others, like the pesticides, are insoluble but sorb rapidly to suspended particles and thus would remain in suspension. Thus, from an experimental viewpoint a variety of test hazardous materials was used with respect to physical/chemical nature after spillage. Likewise, the chemical structure of the compounds was variable enough to provide an adequate test of the biological countermeasure.

Selection Based on Literature Review

The literature was reviewed to gather information on the physical/chemical characteristics of these compounds, previous biodegradation and biological treatment investigations, and the toxicity of the compounds to organisms in fresh and marine waters (see Section 6). The literature survey revealed that most of the contract compounds have been shown to be biodegradable and that the breakdown pathway was completely or partially known (Table 3). Of the 20 top-ranked hazardous materials, most were amenable to mitigation by biological countermeasures. Furthermore, the nature of the materials' toxicity to organisms in natural systems had also been defined to some extent, thus the concentrations that must be achieved to mitigate the effects of hazardous materials were also known.

No hazardous materials were deleted following the literature review, but it was evident that certain of the compounds would require special apparatus or special handling according to the University of Texas at Austin Safety Office. Thus, those compounds that could be tested most easily were elevated in priority.

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TABLE 1. CONTRACT LIST OF HAZARDOUS MATERIALS

Hazardous materials	Ranked priority[1]	Contract priority High	Contract priority Low
Phenol	1	x	
Methyl alcohol	2	x	
Cyclic rodenticides[2]	3		x
Acrylonitrile	4	x	
Benzene	6	x	
Misc. cyclic insecticides	8		x
Styrene	10		x
Acetone cyanohydrin	11	x	
Nonyl phenol	13	x	
DDT	14		x
Isoprene	15	x	
Xylenes	16	x	
Nitrophenol	17	x	
Aldrin-Toxaphene group	18	x	
Total Number	14	10	4

[1] Dawson, et. al. (1970).

[2] Includes Dicetel, Endosulfon, Methoxychlor, Parathion, Methyl Parathion, Chlordane, Dieldrin, Endrin, Heptachlor, Terpene, Polychlorinates, Carbopheno Thion, Coumaphos Diazonon, Dioxathion Ronnel, Chlorobenzilate, DDD, and others.

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TABLE 2. PHYSICAL/CHEMICAL CHARACTERISTICS OF CONTRACT COMPOUNDS

Hazardous material	Molecular weight	Density (g/ml)	Solubility (gms/100 ml H_2O)	Formula
Acetone cyanohydrin	85.10	0.932	very soluble	$(CH_3)_2CHCN$
Acrylonitrile	53.06	0.797	soluble	CH_2: CHCN
Aldrin	365		< 0.00001	$C_{12}H_8Cl_6$
Benzene	78.11	0.879	0.082 @ 22°C	C_6H_6
Cyclic rodent & insect.				
DDT	354.50		insoluble	C $Cl_3CH(C_6H_4Cl)_2$
Isoprene	68.11	0.6806	insoluble	CH_2: $CHC(CH_3)$: CH_2
Methyl alcohol	32.04	0.7961	∞	CH_3OH
Nitrophenol	139.11			NO_2C_6H4OH
o-nitrophenol		1.657	0.21 @ 20°C	
m-nitrophenol		1.485	1.35 @ 25°C	
p-nitrophenol		1.479	1.6 @ 25°C	
Nonyl pohenol	220.3	0.94	slightly soluble	$C_6H_4(C_9H_{19})OH$
Phenol	94.11	1.072	6.7 @ 16°C	C_6H_5OH
Styrene	104.14	0.9074	0.032	$C_6H_5CH{:}CH_2$
Xylenes	106.16			$C_6H_4(CH_3)_2$
o-xylene		0.8802	insoluble	
m-xylene		0.8642	insoluble	
p-xylene		0.8610	insoluble	
Toxaphene	413		< 0.0003	$C_{10}H_{10}Cl_8$

TABLE 3. RESULTS OF LITERATURE REVIEW AND SCREENING TESTS

Hazardous material	Biodegradation shown?	Pathway known?	Toxicity defined?	Biodegradation screening tests
Acetone cyanohydrin	Yes[1]	Partially	Yes, as CN	---
Acrylonitrile	Yes	Partially	Yes	---
Aldrin	Yes	Partially	Yes	x
Benzene	Yes	Yes	Yes	x
Cyclic rodent & insect.	Yes[2]	Partially[2]	Yes	---
DDT	Yes	Partially	Yes	---
Isoprene	No[3]	No[3]	Partially	x
Methyl alcohol	Yes	Yes	Yes	x
Nitrophenol	Yes	Partially	Yes	x
Nonly phenol	No	No	Partially	x
Phenol	Yes	Yes	Yes	x
Styrene	No[3]	No[3]	Partially	x
Xylene	Yes	Partially	Yes	x
Toxaphene	No	No	Yes	x

[1] Following chemical dissociation.

[2] For a few compounds.

[3] No direct evidence, but should be biodegradable; probable pathway known.

Initial Screening Tests

The screening tests performed in this study were designed to show that the compounds could be biodegraded with organisms from various sources. As shown in Table 3, screening tests were performed on most of the contract compounds and either biodegradation or volatilization was demonstrated. Methyl alcohol, nitrophenol, and phenol were all biodegradable by the organisms used, while isoprene, benzene, styrene and xylene volatilized rapidly from the test containers. Volatilization tests for benzene were conducted under various mixing conditions ranging from aerated and highly mixed to unstirred. For the other compounds, no biodecomposition was shown or the results were uncertain.

FINAL SELECTION

Following the screening tests and the initiation of growth kinetics tests, it became apparent that only a few compounds could be tested as extensively as desired in the spill simulation tests. Thus, phenol and methanol were selected because they had been shown to be decomposable, their breakdown pathways differed (methanol was a linear chain decomposition, while phenol was ringed), both were soluble in water, and both could be handled safely by laboratory personnel with reasonable precautions. It was concluded that if the methodology of the biological countermeasure could be demonstrated with these compounds, the feasibility of the countermeasure would be demonstrated and the test procedures could then be applied to other, more difficult-to-handle compounds.

SECTION 6

LITERATURE REVIEW

Engineering and scientific literature was surveyed for information on the physical and chemical properties, biodegradability, and toxicity of the contract list of hazardous materials. Though some of this information, such as physical and chemical properties and toxicity, was readily available in handbooks or in previous reports on hazardous materials spills, information on microbial decomposition of these compounds was variable in quantity and was scattered throughout the literature. For the purposes of this study, a compilation of this information was considered necessary to determine which of the contract compounds would be most amenable to microbial decomposition and hence experimentation.

Following are the results of the literature survey for each contract compound and for the specific topics: general physical/chemical properties, microbial decomposition, and toxicity. Other information has been compiled through the literature survey on other topics and is presented elsewhere in the report.

ACETONE CYANOHYDRIN

Description

Acetone cyanohydrin $(CH_2)_2C(OH)CN$ (hydroxyisobutyronitrile, 2-hydroxy-3-methylpropanenitrile, isopropylcyanohydrin) is formed by the reaction of acetone plus hydrogen cyanide and may contain 0.2 percent free hydrogen. It is very soluble in water, alcohol, and ether. Acetone cyanohydrin decomposes rapidly in alkali, releasing HCN.

Physical/Chemical Properties

Physical state:	colorless liquid
Molecular weight:	85.10
Melting point:	-19°C
Boiling point:	82°C at 23 mm Hg
Refractive index:	1.3996
Density:	0.932 (19°C)
Vapor density:	2.95 (air = 1)
Vapor pressure:	0.88 mm Hg at 20°C

Microbial Decomposition

Little direct information could be found regarding the microbial decomposition of acetone cyanohydrin. However, investigations of the toxic cyanide component have been conducted. Lutin (1970) found that unacclimated activated sludge microorgan-

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isms were totally inhibited by 500 ppm cyanide but the effects on bacterial respiration at lower levels of this toxicant were not analyzed.

Moore and Kin (1969) reported that decomposition probably occurred following the spill of acetone cyanohydrin from a train wreck at Dunreith, Indiana. Five tank cars carrying chemicals, two with acetone cyanohydrin, one with ethylene oxide, one with vinyl chloride, and one with methyl methacrylate were involved in the wreck. On-site inspection determined that the ethylene oxide had vaporized and also burned. One tank load of acetone cyanohydrin was primarily intact except for a small loss of the chemical due to volatilization and subsequent burning at the pressure relief valve. The suspected source of stream pollution from the wreck was the 4,560 liters (1,200 gal) of acetone cyanohydrin lost from the other tank car. Estimates of the toxic potential from the 4,560 liters (1,200 gal) of spilled material showed that it contained 1,260 kg (2,800 lb) of cyanide and most of this toxic material found its way to the river. Measured concentrations as high as 405 mg/l cyanide were found in Blue Creek immediately downstream from the site of the wreck. It was determined that aquatic cyanide concentrations were being reduced by decomposition and dilution, but for further protection of fish and other aquatic life, additional chemical oxidation by calcium hypochlorite was employed.

Transformation of cyanide by biochemical means may be estimated as conversion of HCN into formic acid following the reactions suggested by McKinney (1962). The chemical oxidation of cyanide is given by Sawyer and McCarty (1967). The other product of hydrolysis of acetone cyanohydrin, presumably a polyhydroxy alcohol, is attacked fairly readily. Sawyer and McCarty (1967) state that acetone cyanohydrin can be completely degraded by microbial action to form carbon dioxide and water with aldehydes and ketones produced as intermediates.

Toxicity

Acetone cyanohydrin is very soluble in water, but decomposes to acetone and hydrogen cyanide under alkaline conditions. Since the liquid is stable under acid conditions, it is primarily transported in the acid state. Hydrolysis of this compound into hydrogen cyanide represents the predominant toxic mechanism, with the acetone by-product exhibiting a toxicity several orders of magnitude lower than cyanide. Because of this probable environmental behavior, the toxicity of acetone cyanohydrin components rather than of the whole compound will be discussed.

The toxicity of acetone is indicated in the work of Patrick et al., (1968) who found that approximately 11,500 ppm acetone was the 120-hour median tolerance level (TLm) for diatoms. The growth rate of *Nitzschia linearis*, a common diatom in unpolluted waters, was reduced fifty percent at this high acetone concentration. Anderson (1944) reported immobilization of *Daphnia magna* at 9,280 ppm acetone over 16 hrs, with marcosis being reversible. However, Dowden and Bennett (1965) found that 10 ppm acetone was the median tolerance limit for *Daphnia* exposed to this solvent for 24 to 48 hours, this value appears to be a gross underestimate.

Since the more actuely toxic component of acetone cyanohydrin is cyanide, it is instructive to examine data describing–SH toxicity. Adverse effects of cyanide on aquatic invertebrates occur at much lower cyanide concentrations than the 500 ppm concentration found to inhibit bacterial respiration (Lutin 1970). Experiments by Reich (1955) showed that amoeba respiration decreases with an increase in cyanide

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concentration. A 0.005 molar cyanide solution was lethal to these protozoans. In addition, cyanide stimulated the utilization of glucose by a soil amoeba, but inhibited a peptone-utilization enzymatic pathway. For the aquatic snail *Physa heterostropha*, a 0.432 ppm cyanide concentration was acutely toxic to half of the organisms exposed over a 96-hour period (Patrick *et al.*, 1968).

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Wallen *et al.* (1957) separately tested both components of acetone cyanohydrin hydrolysis (acetone and CN^-) on *Gambusia affinis*, the mosquitofish. The TL_m value for a 96-hour exposure to mosquitofish was 1.6 ppm potassium cyanide. This concentration decreased to 0.28 ppm for bluegill sunfish (*Lepomis machrochirus*) exposed for shorter periods (up to 48 hours). The equivalent toxicity for acetone over a 96-hour test exceeded 13,000 ppm for mosquitofish and 14,000 ppm for orange-spotted sunfish (*Lepomis humilis*). However, the vigorous aeration used to maintain high turbidity as a secondary experimental parameter may have enhanced solvent evaporation from the system and inflated the actual toxic level. Since Cairns and Scheier (1968) showed that 50 percent survival occurred at 8,300 ppm acetone for bluegills and because the system concentration decreased by one-half over the 96-hour test period, loss of the volatile solvent may indeed have been a significant factor.

Freshwater fish succumbed to 0.05 to 0.10 ppm cyanide following an Indiana railroad derailment and subsequent spillage of acetone cyanohydrin into local waterways (Moore and Kin, 1969). Cairns and Scheier (1968) reported that 0.07 ppm cyanide (from KCN) allowed 100% survival of bluegills, but 0.18 ppm killed one-half the test fish, and 0.24 ppm killed all sunfish. Previous experimentation on bluegills had established that variations in fish size did not result in significant toxicity differences with cyanide (Cairns and Scheier 1968). However, Turnbull *et al.*, (1954) noticed that 1 ppm cyanide killed bluegills within an hour after violent spasms and loss of equilibrium, but a concentration of 0.21 ppm did not evoke behavioral response or death. Guppies (*Lebistes reticulatus*) required 0.26 ppm (43-hour exposure) to 0.42 ppm cyanide (20 hours) to kill 50 percent of the fish (Chen and Selleck 1968).

Renn (1955) compared the cyanogenic properties of lactonitrile, an organic nitrile that dissociates more readily than acrylonitrile, and potassium cyanide. Both cyanide-producing compounds yielded similar median tolerance values for centrarchid fishes. Yellow-breasted sunfish (*Lepomis autritus*) and largemouth bass (*Micropterus salmoides*) partially succumbed to 0.06 ppm cyanide while the median tolerance levels for bluegills was 0.01 to 0.06 ppm and for crappies (*Pomoxis annularis*) was 0.05 to 0.07 ppm cyanide. These TL_m values decreased for crappies and increased for bluegills under continuous flow bioassay techniques. Burdick *et al.*, (1958) also used a continuous flow apparatus for testing cyanide effects on coldwater fishes, such as brown trout (*Salmo trutta*) and smallmouth bass (*Micropterus dolomieui*). However, they used minimum cyanide concentrations of 0.32 ppm for trout and 0.175 ppm for bass and lethal effects on all fishes were evident within minutes, rather than hours.

ACRYLONITRILE

Description

Acrylonitrile is the compound $H_2C = CHCN$ (vinyl cyanide, 2-propenenitrile, cyanoethylene). Acrylonitrile is miscible with most organic solvents and exhibits a water solubility of 5.35 percent at $20^{\circ}C$.

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Physical Properties

Physical state:	colorless liquid with fairly pungent odor
Molecular weight:	53.06
Boiling point:	77.3°C
Freezing point:	-83.55°C
Specific gravity:	0.8060 at 20°C
Vapor density:	1.83 (air = 1.0)
Viscosity, liquid:	0.34 cp at 24°
Refractive index:	1.3888 @ 25°C

Microbial Decomposition

Only five percent of acrylonitrile by volume is soluble in water, but, because acrylonitrile may undergo hydrolysis upon water contact, toxic action can result from both the compound itself and from the liberated cyanide. Bacterial oxidation of this organic nitrile occurs through the enzymatic hydrolysis of the nitrile to a carboxyl group, producing ammonia and organic acids (Mills and Stack 1955, Buzzell et al., 1968). Ludzack et al., (1959) found that microorganisms capable of nitrile oxidation are common in surface waters. Cocci and rod-shaped bacteria, usually gram-negative, were commonly found; Pseudomonas aerogenes was especially abundant. Buzzell et al., (1968) concluded that 400 ppm acrylonitrile constitutes the mean tolerance level (50 percent inhibition of growth) for mixed bacterial cultures (sewage seed). Cherry et al., (1956) found zoogleal and algal growth in natural river water with 10 and 25 ppm acrylonitrile added. However, at 50 ppm fungi were the major component of the biota in microcosm simulations.

Lank and Wallace (1970) have tested acrylonitrile under anaerobic conditions and claim that a digester can receive 20 mg/l without adversely affecting its performance.

One important factor affecting degradability is the known volatility of acrylonitrile. Buzzell et al., (1969) subjected a sample of acrylonitrile to a stripping test and found that 50 percent of the carbon was removed after 2 hours and 88 percent was removed after 6 hours. Accordingly, they reasoned that physical stripping was responsible for the carbon loss when experimental samples were subjected to shade-table tests.

Toxicity

Mammals--

Acrylonitrile is toxic to humans and other mammals by inhalation of vapors, ingestion, or skin contact. However, specific expsosure levels for humans have not been documented. Current occupational safety levels are based on analogy to complete hydrolysis of acrylonitrile to HCN, but cyanide formation as the predominant mode of toxicity is still a debatable point. Brieger et al., (1952) found small quantities of HCN in the blood of rats expossed to 100 ppm acrylonitrile vapors, but detected none at lower atmospheric concentrations. However, other investigators (Barnes and Cerna, 1959; Paulet and Desnos 1961, and Paulet et al., 1966) conclude that the whole molecule, not liberated HCN, is responsible for the toxic action.

Excretion of slight amounts of acrylonitrile in the urine has been noted (Czaikowska, 1971), but higher thiocyanate levels in blood serum and urine suggested this route for elimination of partially-metabolized acrylonitrile (Lawton *et al.*, 1943; Paulet and Desnos, 1961).

Acrylonitrile forms conjugates with protein and non-protein sulfhydryl groups, decreasing tissue levels of essential amino acids, such as L-cysteine and L-glutathione (Hashimoto and Kanai, 1965 and 1972). Inhibition of cytochrome oxidase in liver, kidney, and brain tissues results from acrylonitrile poisoning (Tarkowski, 1968) and this blockage of cellular metabolism may explain the accumulation of pyruvate and lactate in acutely intoxicated animals (Hashimoto and Kani, 1972). Acrylonitrile may damage the synthesizing function of the liver, thereby upsetting the balance of available amino acids for growth and maintenance (Movsumzade, 1970).

Aquatic Organisms--

Several investigations of acrylonitrile toxicity to fishes have been reported. For the marine pinfish (*Lagodon rhomboides*), the 24 hour-TL_m was found to be 24.5 ppm (Garrett, 1957). Renn (1955) had previously determined that pinfish could tolerate 10 to 18 ppm acrylonitrile (as acrylonitrile-N) without adverse effects. Buzzell *et al.*, (1968) found that 10 ppm represented the maximum concentration for 100 percent survival of bluegills. Bandt (1953) reported 100 ppm acrylonitrile as the TL_m value for fish, a significantly higher figure than previously determined.

Henderson *et al.*, (1961) found that median tolerance values decreased considerably with length of exposure for fathead minnows (*Pimephales promelas*), bluegills (*Lepomis macrochirus*), and guppies (*Lebistes reticulatus*). Acrylonitrile levels of 34.3 ppm (fatheads), 25.5 ppm (bluegills), and 44.6 ppm (guppies) were acutely toxic to one-half the test fishes within 24 hours and thus demonstrated interspecific differences in toxic susceptibility. The same authors also noted a high cumulative (chronic) toxicity for fathead minnows in a continuous flow system. The TL_m threshold decreased from 33.5 ppm after a one-day exposure to 2.6 ppm for fish exposed to acrylonitrile for 30 days. Tainting of fish flesh by acrylonitrile did not occur at sublethal concentrations.

ALDRIN

Description

Aldrin is a broad-spectrum cyclodiene insecticide of the group that includes chlordane, endrin, dieldrin, and heptachlor. This compound (1,2,3,4,10,10-hexachloro-1,4,4,1,5,8,8a-hexahydro-1, 4-endo, exo-5, 8-dimethanonapthalene) possesses a melting point of 104°C and is most soluble in aromatic hydrocarbons and carbon tetrachloride. Aldrin has unsubstituted double bonds that readily add oxygen to form epoxy derivatives. Upon epoxidation in sunlight, dieldrin is formed. Epoxides are also formed in tissues (both plant and animals) and are preferentially concentrated and stored in fats.

Microbial Decomposition

Aldrin has been shown to be degradable by several mechanisms: microbial decomposition, photochemical oxidation, and volatilization. Kearney and Kaufman (1972) reported that aldrin was oxidized to dieldrin by a number of soil microorganisms including species of Trichderna, Fusarian, and Penicillum. Similar microbial conversions have been reported by Gakstatter (1968) and Krieger and Lee (1973).

Adsorption of aldrin by floc-forming bacteria isolated from Lake Erie has been reported by Leshniowsky et al., (1970). A gram-positive Bacillus species and gram-negative Flavobacterium concentrated aldrin from colloidal suspension and removed it from the water phase upon settling. Apparently, loss of aldrin from the test solution (1 ppm aldrin) resulted solely from adsorption of the pesticide to the organic floc and not from any microbial metabolism. This transport mechanism appears to be highly significant since most chlorinated pesticide biodegration occurs under anaerobic conditions, which are likely to occur in the sediments. Hill and McCarty (1967) reported on the anaerobic degradation of aldrin (where, by definition, degradation referred to any measureable change in the pesticide concentration). They reported that the anaerobic degradation of aldrin by both thick and diluted, digested wastewater sludge suggested first-order kinetics.

Crosby (1972) stated that epoxidation in air can result in the formation of dieldrin when aldrin is exposed to the ultraviolet component of sunlight. Similar photoconversions have been demonstrated by Rose and Sutherland (1967) and Georgacakis and Khan (1971).

Lichtenstein (1972) stated that volatilization was directly responsible for the loss of pesticide residues from a given substrate. In particular, aldrin was the most volatile of eight different insecticides tested.

Toxicity to Aquatic Organisms

Because the decomposition products of aldrin are highly toxic, the overall problem of aldrin toxicity to aquatic life also includes the toxic metabolites and photodecomposition products. Thus, aldrin and its conversion products have been tested for their toxicity effects on numerous fishes and food-chain invertebrates.

Relatively few studies of aldrin toxicity to microorganisms and algae have been conducted in comparison to the number of studies on invertebrates and fishes. Poorman (1973) showed that aldrin levels of 50 to 100 ppm reduced cell numbers of the photosynthetic Euglena gracilis by 12 to 17 percent, but this species encysted and recovered when high pesticide levels were significantly diluted .

Adverse effects on invertebrates occur at much lower aldrin concentrations than noted for planktonic species. Anderson (1960) noted that 29.2 ppb immobilized Daphnia magna, while Sanders and Cope (1966) verified this by demonstrating that Daphnia swimming ability was indeed inhibited at 30 ppb. Only 1 ppm aldrin induced total mortality in lumnacid snails (Batte et al., 1951). Freshwater amphipods (Gammarus lacustris) exhibited a 48-hour TL_m value of 38.5 ppm aldrin (Gaufin et al., 1965; Nebeker and Gaufin, 1964), although McDonald (1962) had found that 50 percent mortality for this species exposed to aldrin for 3 to 4 hours occurred at only 0.5 ppm. Paleomonetes kadiakensis exhibited differential toxicity resistance to aldrin, depending on whether these small freshwater shrimp were collected from agricultural areas (185 ppm) or from wildlife refuge areas free from extensive pesticide usage (85 ppm). Aquatic insect naiads (Acroneuria pacifica, Pteronarcys californica) appeared to be susceptible to less than 0.2 ppm aldrin (Jensen and Gaufin, 1964; Moye and Luckmann, 1964; Gaufin et al., 1965; Jessen and Gaufin, 1966). Because of its economic importance as a food organism in Louisiana, crayfish (Procambarus clarkii) are often grown in flooded rice fields subject to frequent pesticide applications. Since crayfish feed predominantly on organic detritus, pesticides adsorbed to this organic matter may be ingested and reach toxic levels in the organism. Aldrin concentrations of 0.038 ppm were shown to be toxic to juvenile crayfish, while 0.6 ppm caused mortality in the adult crustaceans (Hendrick and Everett, 1965). Other commercially important organisms, especially molluscan filter-feeders (oysters, clams, mussels) have been shown to concentrate aldrin hundreds of times over the ambient water level (Butler, 1967; Casper, 1967; Bedford et al., 1968).

The list of fishes that have been bioassayed for aldrin toxicity is quite large. Aldrin levels of 5.2 to 60 ppb have been shown to be toxic to bluegill sunfish in 96-hour toxicity bioassays (Tarzwell, 1959; Henderson et al., 1959; Weiss, 1964; Ferguson et al., 1964; Profitt, 1966). However, Cope (1963) claimed a 10 ppm TL_m for bluegills exposed only 24 hours. A decrease in median tolerance limit from 9.7 ppm to 5.6 ppm accompanied a temperature increase of 45° to 85°F for bluegills (Cope, 1965). Other centrarchids, such as the green sunfish (Lepomis cyanellus) and the largemouth bass (Micropterus salmoides), exhibited identical TL_m values of 0.4 ppm (Profitt, 1966).

Tarzwell (1959) and Henderson et al. (1959) showed that aldrin toxicity, as indicated by 96-hour TL_m values, was 32 to 33 ppb for fathead minnows, 28 to 30 ppb for goldfish, and 30 to 33 ppb for guppies. Gakstatter (1968) found that radio-labeled aldrin (^{14}C-aldrin) was toxic to goldfish at 0.05 ppm and that conversion of this pesticide to dieldrin proceeded rapidly in the body tissues, but more slowly in the visceral fat. The mosquitofish, Gambusia affinis, was adversely affected by aldrin levels of 0.05 to 2.1 ppm (Boyd and Ferguson, 1964), 0,5 ppm (Mulla et al., 1963), and 0.02 to 0.06 ppm (Ferguson, Cully et al., 1965).

Profitt (1966) demonstrated interspecific variation in susceptibility to aldrin for Notropis minnows, with TL_m values of 0.02 to 0.4 ppm for N. umbratilis, 0.02 to 0.08 ppm for N. cornutus, and 0.6 ppm for N. blennius. The 50 percent survival level was 0.08 ppm for Notemigoneus chrysoleucas, (common golden shiner) and 0.013 to 0.185 ppm for Ictaluras melas (bullhead catfish) (Ferguson, Cully et al., 1965). Katz (1961) reported that chinook salmon (Oncorhychus tshawytsha) found 7.5 ppb toxic, while toxicity effects occurred at 17.7 ppb for rainbow trout (Salmo gairdnerii) and 45.0 ppb for Coho Salmon (0. kisutch). Cope (1965) determined a slightly higher TL_m of 31 ppb to rainbow trout.

BENZENE

Description

Benzene is a cyclic hydrocarbon (C_6H_6) that is colorless, volatile, and possesses a distinct aromatic odor. The chemical structure consists of alternating unsaturated bonds that create a stable resonating ring. Because it undergoes numerous substitution reactions without breaking the ring structure, benzene serves as the parent compound for many other industrially important aromatic hydrocarbons.

Benezene is miscible with most organic solvents, particularly alcohol and ether. Water solubility is fairly low--0.057 percent by weight at 20°C. However, benzene forms a two-phase azeotropic mixture with water which boils at 69.25°C compared to 80.103°C for the pure compound. Using gas chromatographic techniques, McAuliffe

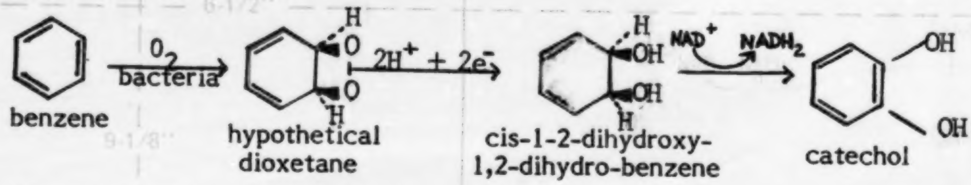
(1963) found that benzene solubility in water was 1,780 ppm at 25°C, highest among all C_1-C_9 hydrocarbons.

Physical/Chemical Properties

Molecular weight:	78.11
Freezing point:	5.506°C
Boiling point:	80.103°C
Density:	0.87903 at 20°C

Microbial Decomposition

The microbial degradation pathway of benzene has been the subject of extensive investigation in recent years. Gibson (1972) has proposed the following reaction sequence for the initial formation of catechol from benzene by the bacteria Pseudomonas putida.

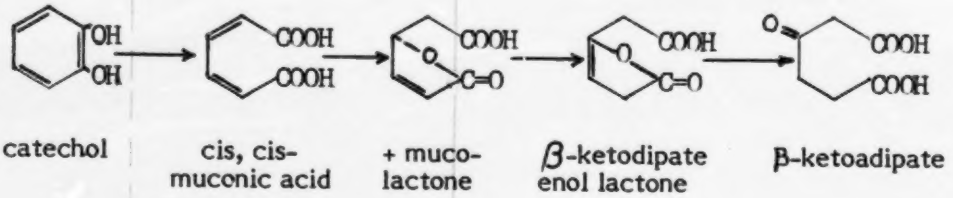


benzene
O_2
bacteria
H
O
O
H
$2H^+ + 2e^-$
hypothetical dioxetane
H
OH
OH
H
NAD^+
$NADH_2$
cis-1-2-dihydroxy-1,2-dihydro-benzene
OH
OH
catechol

According to Dagley (1972), the unsubstituted benzene nucleus is an inert resource structure suitably substituted by two hydroxyl groups. The catechol produced during microbial metabolism is available for enzymic ring fission because of the two hydroxyl groups in the ortho position.

Catechol and its carboxylated derivative, protocatechuic acid, can be catabolized by either meta or ortho cleavage (Chapman, 1972). Ortho cleavage results in fission of the bond between the two carbon atoms bearing the hydroxyl groups. In meta cleavage, fission, occurs between a carbon attached to a hydroxyl group and a carbon atom bonded to hydrogen. In ortho cleavage, the benzene derivative is converted into dicarboxylic acid, whereas meta cleavage results in either an aldehydo-acid or a keto-acid.

Gibson (1968) listed the following sequence as typifying the ortho cleavage of catechol by Pseudomonas putida.



OH
OH
catechol
COOH
COOH
cis, cis-muconic acid
COOH
O
C=O
+ muco-lactone
COOH
O
C=O
β-ketodipate enol lactone
O
COOH
COOH
β-ketoadipate

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The B-ketoadipate thus formed is readily available for further decomposition into succinate and acetyl-CoA, both of which can enter into the tricarboxylic acid cycle with production of CO_2 and H_2O.

Ater adding benzene to a Warburg respirometer, Marr and Stone (1961) detected catechol chromatographically in culture filtrates of *Pseudomonas aeruginosa* and *Mycobacterium rhodochrous* adsorbed onto silica gel. The amount of benzene added could be determined by weighing the silica gel before and after adsorption. Gibson (1972), as stated earlier, found that *Pseudomonas putida* was capable of attacking benzene. *Pseudomonas putida* would not grow when benzene was added directly to the growth medium. However, good growth did occur when benzene was added in the form of a vapor.

Malaney and McKinney (1966), in their studies of the oxidative abilities of benzene-acclimated activated sludge, found that in 20 days it was possible to acclimate normal activated sludge to utilize 250 mg/l benzene as the sole carbon source. By observing pressure changes in the absence of sludge, they concluded that a change in benzene concentration did not occur as a result of volatility. Presumably, conditions did not include aeration. The bacterial species isolated from the sludge were tentatively identified as *Flavobacterium lactis*, *Achromiter sulfurem*, *Achromobacter superficiale*, *Alcaligenes marshalii* and *Rhizobium lupini*.

Toxicity

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Plants--

Kauss *et al*., (1973) observed the reduction in growth of *Chlorella vulgaris* when the organism was exposed to various concentrations of benzene. At 25 ppm benzene, a 15 percent reduction in algal growth was noted after one day, but recovery was complete within six days. A pattern of growth depression in proportion to benzene concentration and the recovery to nearly normal growth rates within 6-10 days was observed at all benzene concentrations below 500 ppm. When benzene concentrations reached 1,000 ppm or more, algal populations did not recover from the benzene addition. Volatilization of benzene likely occurred from the unstoppered flasks used in this experiment, thereby inflating the amounts needed to actually inhibit growth.

Photosynthetic activity of the giant kelp (*Macrocystis pyrifera*) is reportedly not affected by exposure to 10 ppm benzene over a 96-hour period (North *et al*., 1959). The herbicidal effects of benzene and methylated derivatives on terrestrial plants were examined by Currier (1951). From his work with partition coefficients (water to paraffin oil) of benzene, xylene, and other simple aromatics, an inverse relationship between toxicity and compound solubility could be drawn. Currier concluded that cellular penetration is facilitated by increasing the number of methyl groups on the benzene ring, thus, xylene or toluene might be expected to be more toxic to plants than benzene.

Aquatic Organisms--

Distinct chemoreception abnormalities in marine crabs have resulted from exposure to sublethal concentrations of benzene (Kittredge, 1971). Feeding behavior in the lined, shore crab (*Pachygrapsus crassipes*) was inhibited by benzene; however, concentrations required to initiate disruption of chemoreception were not reported.

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Benzene inhibition of crab feeding behavior tended to be more transient than that observed for more complex aromatics, such as napthalene.

Shelford (1917) evaluated the effects of benzene from waste coal tar on freshwater fishes. The small, orange-spotted sunfish (*Lepomis humilis*) used in Shelford's experiments died within one hour of exposure to concentrations of 35 to 37 ppm benzene, preceded by behavioral abnormalities such as erratic movement and intoxication. Median tolerance limits for bluegill sunfish were 20 ppm benzene over 24- and 48-hour exposures (Turnbull *et al.*, 1954). Benzene at 60 ppm killed all test fishes within two hours and was lethal to bluegills within 24 hours at 34 ppm. These investigators calculated that 6 ppm is a safe concentration in regard to acute toxicity for bluegills.

The data of Wallen *et al.*, (1957) appears grossly distorted due to air stripping of benzene from the experimental tanks. All *Gambusia affinis* survived in benzene concentrations of 300 ppm, 10 times the lethal threshold described by other investigators. TL_m values of 395 ppm (24- or 48-hr exposure) and 386 ppm (96-hour exposure) for the mosquito-fish were reported, with a dose of 1,000 ppm benzene killing all fish within 16 minutes. Black bullheads (*Ictalurus melas*) maintained a 1,580 ppm median tolerance limit (48-hour test), but this value decreased to 780 ppm when a 40 percent benzene-acetone mixture was introduced to insure greater benzene solubility.

Acute toxicity of benzene to several freshwater fishes was investigated by Pickering and Henderson (1966). Bluegills exhibited a TL_m of 22.5 ppm for all exposure intervals, almost precisely the same toxic concentration previously derived by Turnbull *et al.* (1954). Fathead minnows (*Pimephales promelas*) tolerated benzene slightly better in hard water (34.4 ppm TL_m) than soft water (35.5 ppm TL_m). Goldfish (*Carassius auritus*) and guppies (*Lebistes reticulatus*) exhibited 24-hour TL_m values of 34.4 ppm and 36.6 ppm, respectively. The volatile nature of benzene may be reflected in the fact that TL_m values for each fish species remained the same throughout 24-, 48- and 96-hour exposure periods.

Measurements of the respiratory stress exhibited by chinook salmon (*Oncorhynchus tshawytacha*) and striped bass (*Morone saxatilis*) when exposed to sublethal benzene concentrations were recorded by Brocksen and Bailey (1973). Since 15 ppm benzene initiated mortality among these juvenile fishes, levels of 5 and 10 ppm were utilized as experimental conditions. For the salmon, 5 ppm benzene increased respiration by 90 percent after 48 hours of exposure, while 10 ppm caused a mean respiration rate increase of 115 percent. The respiration pattern differed significantly for the striped bass, with increases reaching 50 percent at 5 ppm and only 15 percent at 10 ppm over a 96-hour test period. Narcosis and related depression of respiration below normal levels was evident, but recovery proceeded fairly rapidly after current velocities in the respirometer were increased.

Brocksen and Bailey (1973) postulated that the differences in the lipid content of the fish body fat were responsible for the different respiratory responses observed for the two species. After benzene is absorbed across the gill surface into the blood, it becomes attached to erythrocytes and lipoprotein for transport to lipid-rich tissues. Metabolism by the liver, kidney, and various body tissues converts some benzene to phenol, while smaller portions are excreted across the gills unchanged. Overloading of the transport and breakdown mechanisms leads to a buildup of benzene, which accumulates in the lipid rich nervous tissue and induces narcosis.

Benzene at concentrations of 400 ppm did not produce avoidance responses in green sunfish (Lepomis cyanellus) (Summerfelt and Lewis, 1967). A benzeneacetone mixture failed to repel the sunfish from the area where the aromatic hydrocarbon mixture was added. Benzene had initially been selected for study due to Shelford's observations (1917) that it was avoided by freshwater fish.

ISOPRENE

Description

Isoprene is a colorless, volatile liquid that is a basic component of natural rubber polymers. The chemical structure is $CH_2=C(CH_3)CH=CH_2$. Although isoprene is soluble in most common hydrocarbons and forms azeotropes with various organic solvents, the substance is considered insoluble in water.

Isoprene can be dispersed in water by the action of soap and emulsifiers and the resultant emulsion can be polymerized by the use of free radical initiators. Isoprene forms peroxides when exposed to air in the absence of inhibitor substances (e.g., hydroquinone, tert-butyl catechol). Polymerized peroxides result in a "gummy" mass.

Physical/Chemical Properties

Boiling point:	$34.067^{\circ}C$
Freezing point:	$-145.95^{\circ}C$
Density:	0.68095 g/ml at $20^{\circ}C$
Refractive index:	1.42194 at $20^{\circ}C$

Microbial Decomposition

No specific reports on the degradation of isoprene were located. The volatile nature of the pure liquid and the "gummy" peroxides produced by aeration would surely hinder the application of most biodegradation techniques.

Toxicity

Pickering and Henderson (1966) evaluated the toxicity of practical grade isoprene to four species of freshwater fish. Bluegill sunfish with a 42.5 ppm TL_m were the most susceptible, while the TL_m was 86.5 ppm for fathead minnows, 180 ppm for goldfish, and 240 ppm for guppies. No variation between exposures of 24, 48 and 96 hours existed. In addition, isoprene toxicity to fathead minnows of different ages was analyzed. Median tolerance limits of 75 to 85 ppm isoprene were found for minnow fry (1 and 2 days old), juveniles (10 days old), and adults. No significant variation between age and toxic susceptibility was indicated.

METHANOL

General Description

At room temperature, methanol (CH_3OH) is a colorless neutral liquid. It is infinitely soluble in water, although the density and viscosity constants for a methanol-water mixture change with temperature and proportion of alcohol (Woodward, 1967).

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Physical/Chemical Properties

Boiling point:	64.7°C
Freezing Point:	-97.8°C
Density:	0.79609 g/ml at 15°C
Refractive index:	1.3287 at 20°C
Viscosity:	0.5945 cP at 20°C

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Microbial Decomposition

Alcaligenes faecalis, a bacterium isolated from activated sludge, has demonstrated the ability to metabolize methyl alcohol (500 ppm) and numerous other aliphatic organic compounds (Marion and Malaney, 1963). An anaerobic microorganism, *Methanosarcina barkeri*, ferments methanol to methane and carbon dioxide (Toenniessen and Mah, 1971; Bryant *et al.*, 1971), but such mechanisms may be of little importance in natural systems. Numerous investigators have reported rapid degradation of methanol by microorganisms in activated sewage sludge (McKinney and Jeris, 1955; Dickerson *et al.*, 1955; Hatfield, 1957). Previously, Placak and Ruchhoft (1947) noted a decrease in activated sludge exposed to methanol and minimal degradation of the compound.

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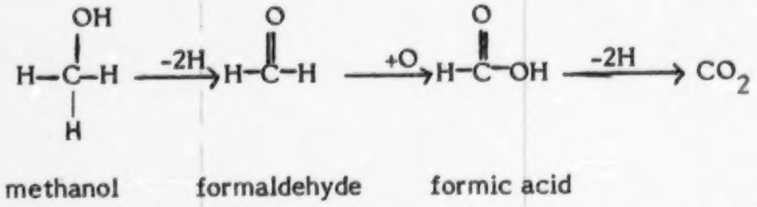
The metabolic pathways for methanol proposed by McKinney and Jeris (1955) provide for conversion of alcohol to aldehyde and then to organic acid. The organic acid is introduced into the tricarboxylic acid cycle and is oxidized to carbon dioxide and water. Stanier *et al.*, (1970) reported that methanol can serve as a growth substrate for *Methanomonas methanica*, producing allulose phosphate as the principal intermediate. Bacteria that can utilize methanol and not methane include various *Pseudomonas* and *Hyphomicrobium* species. An analogous pathway to that used by *Methanomonas methanica* was utilized with pyruvic acid produced as the intermediate.

Although there were questions regarding whether methanol follows the oxidation pathway of other primary alcohols (McKinney and Jeris, 1955; Marion and Malaney, 1963), numerous recent works confirm that bacteria that grow on methane or methanol as a source of carbon and energy, oxidize methane, methanol, formaldehyde, and formate to carbon dioxide as follows (Patel and Hoare, 1971; Brown *et al.*, 1964; Dworkin and Forster, 1956; Anthony and Zatman, 1965; Anthony and Zatman, 1967a, 1967b; Hepstinsall and Quayle, 1970):



$$\mathrm{H{-}\overset{OH}{\underset{H}{C}}{-}H} \xrightarrow{-2H} \mathrm{H{-}\overset{O}{\overset{\|}{C}}{-}H} \xrightarrow{+O} \mathrm{H{-}\overset{O}{\overset{\|}{C}}{-}OH} \xrightarrow{-2H} CO_2$$

Oxidation of methanol and formaldehyde is known to be catalyzed by a nonspecific primary alcohol dehydrogenase (PAD) that is activated by ammonium ions. Bacteria reported to have this ability include *Alcaligenes faecalis* (Marion and Malaney, 1963), *Methylococcus capulatus* (Patel and Hoare, 1971), *Methanomonas methanooxidans*

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(Brown *et al.*, 1964) and various *Pseudomonas* species. (Dworkin and Forster, 1956; Anthony and Zatman, 1965, 1967a, 1967b; Heptinstall and Quayle, 1970).

Toxicity

Mammalian Toxicity--

Methyl alcohol exerts its toxic action on mammalian systems primarily upon ingestion. Only 9.1 mg methanol/kg of body weight has been reported as the acute oral toxicity level for rats (Welch and Slocum, 1943). Ingestion of this toxic alcohol by humans may lead to blindness and even death. Metabolism of methanol by microbes did not appear to follow the normal oxidation pattern of alcohol to aldehyde to organic acid (McKinney and Jeris, 1955), but if mammalian systems follow the normal pathway, the resultant metabolite, formaldehyde, may be the toxic agent.

Aquatic Organisms--

Although methanol exhibits an almost infinite solubility in water, aquatic organisms appear extremely tolerant of this low molecular weight alcohol. Anderson (1944) reported on the threshold concentrations of methanol required to narcotize or immobilize *Daphnia magna*. A one-molar solution of methanol (32,000 ppm) inhibited swimming of these cladocerans in Lake Erie water. Methanol exhibited the highest threshold concentration of all salts and organic compounds tested. Stimulation of movement in water beetles (*Laccophilus maculosus*) exposed to various anions, cations, and simple organics was the reaction threshold tested by Hodgson (1951). Methanol concentrations of 3.6 moles (115,000 ppm) elicited movement by 50 percent of the beetles. This extremely high chemotaxic threshold is 190 times higher than the detection threshold for the barnacle *Balanus* (Cole and Allison, 1930), but three times less than the sensitivity level for the *blowfly Phormia* (Dethier and Chadwick, 1947).

The creek chub (*Semotilus atromaculatus*) in Detroit River water exhibited a critical toxicity range of 8,000 to 17,000 ppm for methanol (Gillette *et al.*, 1952). This toxicity level indicated the acute threshold above which all test fishes died and below which all survived for 24 hours. A typical TL_m value for this type of short-term experimental test should fall near the middle of this critical range. McKee and Wolfe (1963) cited data previously reported showing that 8,100 ppm methanol did not injure fingerling trout from natural waters in 24 hours. In addition, adult trout tolerated 10,000 ppm for two hours without adverse effects.

NITROPHENOL

General Description

Nitrophenol (ortho, meta, and paraisomers) occurs in phenol-acclimated cultures, but at a substantially reduced rate in comparison to most other phenol and benzene derivatives (Chambers *et al.*, 1963). Oxygen uptake by microorganisms exposed to 100 ppm nitrophenol was greatest for m-nitrophenol, followed by o-nitrophenol, and then p-nitrophenol at slightly lower rates. Metabolic pathways suggesting the removal of the nitro group as nitrite before ring cleavage have been postulated (Simpson and Evans, 1953), but some investigators believe that benzene ring cleavage occurs before the nitro group is eliminated. Still, the abilities of natural microbial populations to metabolize the nitrophenols and the enzymatic pathways by which this is accomplished are largely unknown.

Toxicity to Aquatic Organisms

Applegate et al., (1957) reported that 5 ppm o-nitrophenol and p-nitrophenol were not lethal to freshwater trout, bluegills, or lamprey larvae. The 24-hour TL_m value for bluegills exposed to o-nitrophenol was 66.9 ppm, while 46.3 to 51.6 ppm o-nitrophenol caused 50 percent mortality in 48-hour tests (Lammering and Burbank, 1961). Chronic toxicity may be significant below 50 ppm for longer exposure periods; in fact, Applegate et al., (1957) implied that 37 ppm might be a typical 96-hour TL_m value. Loss of equilibrium by the bluegills preceeded death for most nitrophenol concentrations but some recovery was noted at sublethal concentrations.

Bringmann and Kuhn (1959) tested several food-chain organisms from the Havel River (Germany) for their toxicity thresholds to the nitrophenols. Daphnia exhibited adverse effects to 14 ppm p-nitrophenol, 24 ppm m-nitrophenol, and 60 ppm o-nitrophenol. Microregma, a protozoan, reacted equally towards m-nitrophenol and o-nitrophenol (20 ppm) and did not exhibit negative effects until 40 ppm o-nitrophenol was reached. The green algae, Scenedesmus, was most tolerant to p-nitrophenol (72 ppm) and less tolerant to o-nitrophenol (36 ppm) and m-nitrophenol (28 ppm). More than 1,000 ppm o-nitrophenol was required to inhibit Escherischia coli, although the bacteria responded to 300 ppm of m-nitrophenol and 100 ppm of p-nitrophenol.

NONYL PHENOL

General Description

Nonyl phenol -- $C_6H_4(C_9H_{19})OH$ -- is a clear, yellow, viscous liquid with a slightly phenolic odor and is used in chemical manufacturing detergents, oil additives, and rubber chemicals. It is very slightly soluble in water, but it is soluble in benzene, ether, carbon tetrachloride, alcohol, and acetone.

Physical Characteristics

Molecular weight:	220.3
Specific gravity:	0.94 at 20°C
Distillation range:	279-301°C
Flash point:	140°F
Boiling point:	300°C

Microbial Decomposition

Nonyl phenol is a phenol ring attached to a chain of 9 carbon atoms. The literature review did not reveal any specific articles on microbial decomposition of this material; however, McKenna (1972) reported on microbial metabolism of a benzene ring attached to a 10-carbon chain. Apparently, good growth was obtained in a number of bacterial strains; however, it was not obvious whether the chain or the ring compound was attacked first. Chapman (1972) stated that a chain containing 3 or more carbon atoms can undergo oxidation provided that extensive branching is not present.

Toxicity to Aquatic Organisms

No information on the toxicity of nonyl phenol to aquatic microorganisms and invertebrates was found. However, experimentation with larval sea lampreys (*Petromyzon marinus*), rainbow trout (*Salmo gairdnerii*), and bluegills has shown that 5 ppm of this alkylated phenol causes death or "obvious distress" to the fishes (Applegate *et al.*, 1957). This experimental level of nonylphenol caused trout to succumb within four hours, while bluegills and lamprey larvae survived for 14 hours before dying. Marchetti (1965) showed that 5.2 ppm of nonyl phenol ethoxylate, a surfactant derivative of nonyl phenol, was toxic to juvenile rainbow trout (40 and 210 days old) within 6 hours. Newly hatched trout alevins could tolerate 42 ppm, but after absorption of the yolk sac the fry found 2.5 ppm to be lethal.

PHENOL

Description

Phenol (hydroxy-benzene) has a single hydroxyl group attached to a benzene ring. Phenol is crystalline when pure and stored at room temperature. However, the commercial grade ("liquified phenol") is either 80 to 82 percent pure phenol, with the remainder being cresols and water. Phenol is water soluble (82 gm/l at 15°C), although its solubility varies with temperature up to 65.3°C. Above this temperature, it is miscible in water in all proportions.

Physical/Chemical Properties

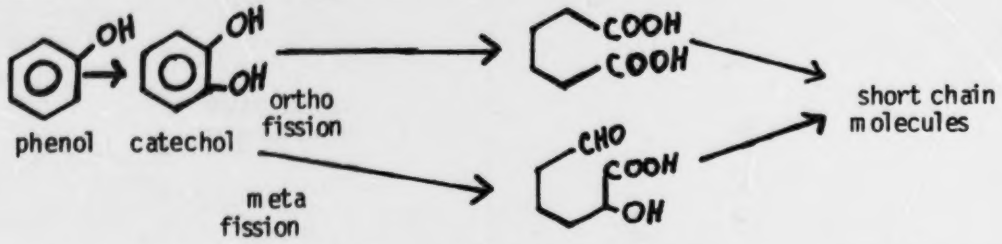
Molecular weight:	94.11
Boiling point:	181.75°C
Freezing point:	40.9°C
Specific Gravity:	1.07 at 20°C

Microbial Decomposition

Phenol is thought to be transformed directly into catechol (dihydroxybenzene). The transformation of catechol into CO_2 and H_2O follows the ring cleavage scheme described previously in the section on benzene. According to Chapman (1972), the microorganism that has been utilized to demonstrate this particular pathway is *Pseudomonas putida*.

Hermann (1959) found that 1,600 ppm phenol was inhibitory to sewage microorganisms. At this level, oxygen utilization by the sewage bacteria was halved. Over 1,000 ppm phenol was necessary to produce threshold toxicity effects on *E. coli*, the common sewage bacterium (Bringmann and Kuhn, 1959). Lutin (1970) demonstrated that 500 ppm phenol exhibited toxic action on some unacclimated sewage sludge; however, mixed bacterial cultures that had been acclimated to 250 to 500 ppm phenol were shown to degrade phenol as well as numerous phenol and benzene derivatives (McKinney *et al.*, 1956; Chambers *et al.*, 1963). Previously, Evans (1947) isolated numerous soil microorganisms that possessed phenol-metabolizing abilities, further indicating the widespread environmental distribution of phenol-tolerant microorganisms.

Evans (1947) proposed that dihydroxyphenol (catechol) was the metabolic intermediate in the biological decomposition of phenol because it was detected in the culture fluid. Based on the concept of simultaneous adaptation, which means that bacterial adaptation to a single compound results in adaptation to its metabolic intermediates, Stanier (1950) reported a widely accepted phenol decomposition pattern. The first step of phenol oxidation is catechol formation by tyrosinase. Catechol is further converted to either aldehydo-acid or ketoacid, which is converted to carbon dioxide and cell protoplasm:



Bacteria reported to have this ability include: Mycobacterium crystallophagum, Micrococcus sphaeroides, Vibrio cuneata (Evans, 1947) and various Pseudomonas species (Evans, 1947; Stanier, 1950; McKinney et al., 1956; Chambers et al., 1963).

Toxicity to Aquatic Organisms

Phenol and a host of phenol derivates have been extensively studied as major pollutants with adverse effects on aquatic organisms. Since toxicity of these phenolic compounds is generally cumulative and often synergistic, the observed environmental disruption from phenolic waste discharge cannot be ascribed solely to phenol itself. Biorefractory (taste and odor-causing) problems are an important sublethal effect of phenolic compounds, especially for freshwater fish in which partitioning through the skin and the resultant tainting of fish flesh commonly occur in phenol-contaminated waters.

Phenol-containing wastewater was apparently responsible for alteration of the "flora and fauna" in a Luxembourg river (Krombach and Barthel, 1963). Destruction of the aquatic community was apparent in areas containing 10 ppm phenol. However, presence of other toxicants in the waste may have contributed to the toxic effects observed. When phenol was applied as a mosquito larvacide, differential survival of fish and Anopheles (mosquito) larvae revealed that vertebrates may be more susceptible to phenol than lower food-chain organisms (Knowles et al., 1941).

The green alga Scenedesmus reached its toxicity threshold concentration at 40 ppm phenol (Bringmann and Kuhn, 1959), while the diatom Navicula linearis required 258 ppm phenol to show a 50 percent reduction in growth rate over 120 hours (Patrick et al., 1968). The phycotoxic action of phenol has been attributed to its tendency to form compounds within the cytoplasm of Ankistrodesmus and Scenedesmus (Keyna, 1940). For the giant marine kelp Macrocystis pyrifera, one ppm phenol caused no adverse effects, but 10 ppm produced a 50 percent reduction in photosynthetic activity over a four-day exposure (Clendenning and North, 1960).

Ukeles (1962) showed that phenol concentrations above 10 ppm were inhibitory to growth of several algal species, especially flagellated forms (*Monochrysis lutheri* and *Dunaliella*) and non-motile coccoid types (*Protococcus* and *Chlorella*). *Protococcus*, *Chlorella*, and *Dunaliella* maintained approximately 50 percent of the control growth rate at 300 ppm phenol, but populations died off at 500 ppm phenol. The diatom, *Nitzchia closterium*, and *Monochrysis lutheri* remained viable but exhibited no growth at 100 ppm.

Phenol toxicity to aquatic organisms has been documented by several investigators. Bringmann and Kuhn (1959) showed that 30 ppm phenol is the toxicity threshold for the protozoan *Microregma*. *Daphnia magna* were immobilized at 94 ppm phenol in Lake Erie water (Anderson, 1944), but the crustaceans (adult) reached a 24-hour TL_m at 61 ppm and a 48-hour TL_m at 21 ppm (Dowden and Bennett, 1965). Juvenile daphnids responded negatively at lower phenol concentrations (17 ppm TL_m over 24 hours and 7 ppm TL_m for a 48-hour exposure). Sollman (1949) calculated an intermediate TL_m of 28.9 ppm phenol for a 48-hour *Daphnia* exposure. *Gammarus pulex*, *Tubifex* worms, and chironomid larvae were reportedly not affected by a 4.3 ppm phenol level (Liepolt, 1953). Patrick *et al*., (1968) demonstrated that the freshwater snail, *Physa heterostropha*, achieved 50 percent survival over 96 hours at 94 ppm phenol.

Goldfish (*Carrassius auritus*) have been used frequently as experimental subjects for phenol toxicity experiments. Powers (1918) observed that goldfish survived for only 104 minutes in 259 ppm phenol. Twenty-five ppm phenol was apparently not lethal to goldfish, whereas 41.6 ppm resulted in two-thirds mortality and 125 ppm killed all fishes in 8 hours during experimentation by Gersdorff and Smith (1940). Gersdorff (1943) also determined acute phenol toxicity at concentrations of 199 to 1,460 ppm, where survival times for goldfish ranged from 12 to 85 minutes. A miminal lethal dose of phenol, when injected into goldfish tail muscle, was 230 mg phenol/kg of fish body weight (Boni, 1965). Boni also determined that goldfish actively excreted phenol directly without first conjugating the compound. A 44.5 ppm TL_m for goldfish exposed to phenol (96 hours) provides the most reasonable estimate of this compound's acute toxicity (Pickering and Henderson, 1966).

Another fish used extensively for phenol toxicity bioassay is the bluegill, *Lepomis macrochirus*. Trama (1955) determined that 20.5 ppm constituted the 96-hour TL_m for this sunfish. Comparable bioassay investigations of this species determined 96-hour TL_m values of 11.5 ppm (Cairns and Scheier, 1959), 13.5 ppm (Patrick *et al*., 1968), and 26 ppm (Pickering and Henderson, 1966). Shorter-term tests by Dowden and Bennett (1965) found that 10 to 15 ppm phenol was an adequate 24-hour TL_m for bluegills, while Lammering and Burbank (1961) calculated 22.2 ppm phenol as the 48-hour TL_m. Based on these previous bioassays, phenol concentrations of 10 to 25 ppm are acutely toxic to bluegill sunfish, indicating that these organisms have a relatively higher sensitivity to phenol than do goldfish.

The effects of major environmental parameters (salinity, dissolved oxygen concentration and temperature) on the toxicity of phenol to rainbow trout (*Salmo gairdnerii*) have been studied by several British investigators. Brown, Jordan and Tiller (1967) found that the median tolerance limit for phenol doubled with a temperature increase from 6°C to 18°C. Trout acclimated to 20 percent salt water showed a 5.2 ppm TL_m (48-hour test), whereas those trout held in fresh water exhibited a higher TL_m (9.3 ppm) (Brown, Shurben and Fawell, 1967). Lloyd (1961) found that reduction in dissolved oxygen concentration below the air saturation value led to increased phenol toxicity. Phenol in concentrations of 6.5 to 9.6 ppm severely damaged the gill filaments of trout in hard water and resulted in extensive damage to numerous organ systems, particularly the liver, kidney, spleen, skin, small intestine, and ovary (Mitrovic et al., 1968).

Bioassays using freshwater fishes frequently demonstrate interspecific variability with regard to phenol toxicity. During their search for an effective lamprey larvacide, Applegate et al. (1957) noted that 5 ppm phenol was lethal to rainbow trout in 10 hours, but was not toxic to bluegill sunfish or sea lamprey larvae. Ten to twenty ppm phenol was indicated as the critical toxicity range (0 to 100 percent morality) for the creek chub, Semotilus atromaculatus (Gillette et al., 1952). The green sunfish, Lepomis cyanellus, was not adversely affected or even repulsed by phenol levels of 20 ppm (Summerfelt and Lewis, 1967). However, the minnow, Phoximus phoxinus, could not discriminate between phenol concentrations of 4 to 400 ppm and became poisoned by the higher doses (Jones, 1951). Forty-eight hour TL_m tests produced values of 40.6 ppm for fathead minnows (Pickering and Henderson, 1966), 56 ppm for mosquitofish (Wallen et al., 1957), 16.7 ppm for fingerling channel catfish (Clemens and Sneed, 1959), and 49.9 ppm for guppies (Pickering and Henderson, 1966).

STYRENE

Description

Styrene (vinyl benzene) is an unsaturated aromatic compound ($C_6H_5CH=CH_2$) used extensively in plastics production (Coulter et al., 1967). This colorless, flammable liquid undergoes the reactions typical of an unsaturated compound and has been utilized in a host of plastics polymer formulations for this reason. Its water solubility is only 0.032 percent at 25°C, although it is infinitely soluble in most organic solvents. Pure styrene polymerizes slowly at room temperature and more rapidly under warmer conditions. Also, oxygen tends to degrade styrene. Polymerization in storage is retarded by 10 to 15 ppm TBC (tert-butyl catechol).

Physical/Chemical Properties

Boiling point:	145°C
Freezing point:	-30.6°C
Viscosity:	0.763 cP at 20°C
Density:	0.9059 g/cm^3 at 20°C
Refractive index:	1.5467 n_D at 20°C

Microbial Decomposition

The number of investigations in the literature on the microbial degradation of phenol are minimal.This may be due to the almost total consumption of phenol for polystyrene plastics products and the lack of unpolymerized styrene in the waste streams of this industry. Ludzack and Ettinger (1960) referred to experimentation by Pahren on the oxidation of styrene by unacclimated activated sludge. Only eighteen percent of the theoretical oxidation was achieved with 10 ppm vinyl benzene, implying relatively slow biodegradation over the 10-hour test period.

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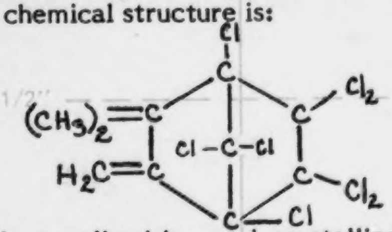
Toxicity to Aquatic Organisms

Literature references to the aquatic toxicity of styrene are minimal. Bioassays of styrene toxicity to fathead minnows, bluegills, goldfish, and guppies were performed by Pickering and Henderson (1966). Ninety-six-hour TL_m values were 25 ppm for bluegills, 64.7 ppm for goldfish, and 74.8 ppm for guppies. Median toxicity was greater for fathead minnows in soft water (46.4 ppm) than in hard water (59.3 ppm). Generally, no variation between 24-, 48- and 96-hour tests occurred, except for small TL_m decreases observed in fathead minnows exposed over longer time periods.

TOXAPHENE

Description

Rather than being a distinct compound, toxaphene ($C_{10}H_{10}Cl_8$) is actually a mixture containing polychloro-bicyclic terpenes. This insecticide is made by chlorinating camphene and its chemical structure is:



The technical product is a yellowish, semi-crystalline gum that contains 67 to 69 percent chlorine and has a melting point of 65° to 90°C. However, it dehydrochlorinates in the presence of alkali, prolonged exposure to sunlight, and at temperatures above 155°C. Toxapheneis generally classified with aldrin as an organochloride pesticide. Like aldrin, it possesses a very low vapor pressure, very low water solubility, and resistance to biological degradation.

The strong tendency of toxaphene to adsorb to various surfaces has been reported in the laboratory as well as in natural systems. Courtenay and Roberts (1973) reported that during their bioassay tests using plastic-lined vessels a substantial portion of the toxaphene was sorbed on the walls of the vessels. In natural systems, Hughes and Lee (1973) reported that environmental persistence is rather complex, since sorption and desorption mechanisms control the presence of this material. Toxaphene transport into lake sediments appears to be a major mechanism for detoxification (Veith and Lee, 1971). This pesticide penetrated lake sediments as deep as 20 cm and resisted subsequent attempts in the laboratory to be leached from the sediments. Also, toxaphene sorbed onto suspended algae and organic particulates and was transported to the sediment surface when this material sank.

Toxicity to Aquatic Organisms

Toxaphene is extremely phytotoxic, with phytoplankton productivity reduced 91 percent when exposed to 1 ppm for 4 hours (Butler, 1963). Concentrations below 0.01 ppm were determined to be sublethal for the green algae Scenedesmus incrassatulus (Schoettger and Olive, 1961). However, Monochrysis lutheri (green marine flagellate) did not grow in 0.00015 ppm toxaphene and growth of Nitzchia closterium (diatom) stopped at 0.04 ppm, but cells remained viable (Ukeles, 1962). Other algal bioassays showed inhibited growth at 0.07 ppm for Chlorella and 0.15 ppm for Protococcus and

Dunaliella. Needham (1966) reported slight declines in blue-green algal species following application of 90 ppb toxaphene to North Dakota lakes.

Application of toxaphene at 100 ppb caused reduction of Tendipedidae on the lake bottom and repopulation took almost a year (Cushing and Olive, 1957; Needham, 1966). Schoettger and Olive (1961) revealed a 0.03 ppm toxicity threshold for both *Daphnia pulex* and *D. magna*, while the *Daphnia pulex* tested by Cope (1966) succumbed to one-half this level (0.015 ppm). Other zooplankton populations in freshwater lakes, especially entomostracans (*Cyclops*, *Ceriodaphnia*, *Diaptomus*), rotarians (*Polyarthia*, *Keratella*, *Asplancha*, *Brachionus*) and protozoans (*Ceratium*, *Difflugia*), declined when exposed to toxaphene, (Hoffman and Olive, 1961; Needham, 1966). However, 100 ppb toxaphene (used to eliminate a fish population) did not permanently alter the lake's invertebrate populations of physid snails, microcrustaceans, and *Chironomus* or *Chaeborus* larval stages (Hilsenhoff, 1965).

The insect naiads (*Pteronarcys californica*, *Pteronarcella badia*, *Claasemia sabulosa*) reacted negatively to 1.3 to 3.0 ppb over a 4 day exposure (Sanders and Cope, 1968). The amphipod *Gammarus lacustris* maintained 50 percent survival in 0.5 ppm toxaphene for only 96 minutes (McDonald, 1962). In comparison, *Paleomonetes kadiakensis* appeared extremely tolerant to toxaphene with 57 to 180 ppm necessary to kill this shrimp (Ferguson, Culley *et al.*, 1965). Toxaphene toxicity to larval crustaceans was greatest during the molting stages. Courtenay and Roberts (1973) used penaeid shrimp and blue crab larval stages to demonstrate maximum toxaphene stress.

Numerous accounts of toxaphene used to eradicate the entire fish population from a lake have been reported. Lawrence (1950) noted that 0.02 ppm was lethal to bluegills, golden shiners, and bass fingerlings in an Alabama pond after two days. Carp, golden shiners, bluegills, yellow perch, bonytail chub, and brown trout were eliminated from several Arizona lakes with 0.1 ppm toxaphene. Restocking was accomplished successfully 9 to 10 months later with rainbow trout (Hemphill, 1954). Fukano and Hooper (1958) stated that a 5 ppb toxaphene concentration could be used to selectively reduce small fish populations (bluegill and pumpkinseed sunfish) and not affect larger fishes (yellow perch, largemouth bass, and rock bass). Roughfish, such as carp and bullheads, could be controlled by 25 ppb toxaphene in Iowa lakes, although excessive turbidity may remove some pesticide by adsorption (Rose, 1958). Stringer and McMynn's (1958) experiments with various British Columbian lakes demonstrated considerable interlake variation of toxaphene toxicity (10-100 ppb) to similar fish fauna. Other investigations of roughfish control by toxaphene application have been conducted in Florida (Huish, 1961), New Mexico (Kallman *et al.*, 1962), Montana (Wollitz, 1963), Alaska (Meehan and Sheridan, 1966), and North Dakota (Warnick, 1966; Henegar, 1966).

Forty-eight-hour TL_m values for bluegills ranged from 3.5 to 4 ppb toxaphene (Tarzwell, 1959; Henderson *et al.*, 1959; Weiss, 1964; Cope, 1966). Ferguson *et al.* (1964) reported pesticide-resistant bluegills that could tolerate 1.6 ppm during short-term bioassays. Fathead minnows exhibited median tolerance limits of 7.5 to 13 ppb (Henderson *et al.*, 1959; Cohen *et al.*, 1961). Earlier, Hooper and Grzenda (1955) had found that toxaphene toxicity was greatest to fathead minnows in hard water, while Schaumberg *et al.* (1967) determined that both high temperature (39°C) and near-freezing levels increased toxaphene toxicity to fatheads.

Gambusia affinis demonstrated that resistance acquired after a single sublethal dose can raise the toxicity level from 0.01 to 0.48 ppm (Boyd and Ferguson, 1964). Bioassays revealed that 0.005 to 0.059 ppm is a normal toxicity range for the mosquitofish (Workman and Neuhold, 1963; Ferguson, Cotton et al., 1965). Guppies tested by Henderson et al. (1959) required 0.02 ppm as the toxic level, but Royer (1966) found only 1 ppb to be lethal to this species. Mahdi (1966) ran bioassays on Notemigoneus crysoleucas, Carassius auratus, Ictaluras melas, and Campostoma anomalum to find median tolerance limits of 12.5-ppb and 94-ppb. The organisms survived up to 2.5 ppb and 14 ppb under the 12.5 and 94 ppb exposure conditions, respectively. Channel catfish fingerlings supposedly survived up to 2.5 ppm toxaphene (Clemens and Sneed, 1959), but bullheads found 0.004 to 0.05 ppm toxic (Ferguson, Cotton et al., 1965). According to Johnson (1966), goldfish required 0.03 to 0.100 ppm of the toxicant before dying.

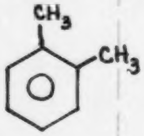
Rainbow trout and other cold-water fishes have often been used as bioassay organisms, since restocking of these gamefish have generally followed roughfish eradication using toxaphene in suitable lakes. Salmo gairdnerii reacted adversely to levels as low as 4 ppb (Cope, 1966) and as high as 54 ppb (Workman and Newhold, 1963). However, most reports show a toxicity range of 8.4 to 16.5 ppb (Katz, 1961; Webb, 1961; Mahdi, 1966). Cope (1965) indicated that temperature increases (45 to 65°F) decrease the amount of toxicant needed to cause mortality. Coho salmon (Oncorhynchus kisutch) found 9.4 ppb as the median tolerance limit, but sockeye salmon (O. nerka) and chinook salmon (O. tschawytscha) reacted to only 3.6 ppb and 2.5 ppb, respectively (Schoettger and Olive, 1961; Katz, 1961).

Courtenay and Roberts (1973) encountered rapid adsorption of toxaphene to plastic-lined bioassay containers, which distorted their TL_{50} values for a group of freshwater and estuarine fishes. They found however, that toxaphene toxicity varied directly with increasing salinity and they postulated that this pesticide interferes with basic osmoregulatory metabolism. For fishes, the most pesticide-sensitive stage was during development of gills in newly-hatched fry.

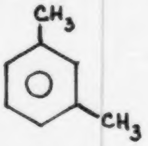
XYLENE

Description

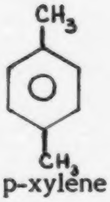
Xylene, an 8-carbon aromatic hydrocarbon, is a dimethyl derivative of benzene. Xylene occurs in three isometric ring forms, as shown below:



o-xylene



m-xylene



p-xylene

Xylenes are generally considered insoluble in water; however, McAuliffe (1963) found that 175 ppm is the approximate water solubility of o-xylene at room temperature. Although this degree of water solubility is only onetenth that of benzene, it is still substantially higher than other alkylated benzenes and aliphatic

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hydrocarbons. m-Xylene forms an azeotrope with water, which contains 64.2 percent m-xylene.

Physical/Chemical Properties

	o-Xylene	m-Xylene	p-Xylene
Boiling point:	144.41°C	139.103°C	138.35°C
Freezing point:	-25.182°C	-47.872°C	-25.182°C
Density (at 20°C):	0.8802 g/ml	0.86417 g/ml	0.86105 g/ml
Refractive index (at 20°C)	1.50545 n_D	1.49722n_D	1.49582 n_D

Microbial Decomposition

The degradation pathway of the three xylene (dimethyl-benzene) isomers is similar to that of benzene and phenol. In mammalian systems, Laham (1970) has shown that various xylenols are produced during metabolism of the xylenes. Chapman (1972) found that Pseudomonas putida demonstrated a similar reaction sequence. He reported that 2, 4-xylenol possesses two methyl groups that are oxidized in succession, yielding protocatechuic acid. The latter compound, which is also a benzene degradation product, is transformed into -ketoadipic acid, an acceptable substrate for the tricarboxylic acid cycle. For the other isomers, 2, 3-xylenol and 3, 4-xylenol, the methyl groups are kept intact to form catechol, which can be broken up via meta cleavage.

Microorganisms in activated sludge have demonstrated degradation of xylene isomers, but the degradation rates differ. When acclimated to aniline (aminobenzene) before experimentation, sludge bacteria metabolized p-xylene the most and m-xylene the least (Malaney, 1960). Activated sludge acclimated to large doses of benzene exhibited significant oxidation of the xylenes. o-Xylenes were degraded the most rapidly and m-xylenes oxidized the most slowly (Malaney and McKinney, 1966). Zoogloea bacteria commonly isolated from polluted natural waters and wastewater streams utilized m-toluate and p-toluate, which are metabolic derivatives of the xylene isomers (Unz and Farrah, 1972).

Nozaka and Kusunose (1968) also found that microbial metabolism of pxylene paralleled mammalian pathways. They determined that Pseudomonas aeruginosa oxidized ^{14}C-labeled p-xylene to p-toluic acid via the corresponding alcohol.

Mammalian Toxicity

None of the three xylene isomers (ortho-, meta-, para-) exhibit the longterm depressive effects on production of red blood cells that results from benzene poisoning. Although experiments with mammals have demonstrated a higher acute toxicity for xylene than benzene, inhibition of the hemopoietic system and resultant aplastic anemia is unproved. Acute effects from inhalation of xylene vapors generally consist of narcosis and unconciousness rather than death (Browning, 1950).

Xylene's role as a commercial solvent has increased substantially due to minimal occupational health dangers in comparison to the other aromatic solvents, benzene and toluene (Browning, 1959). The neurotoxic effect of xylene is greater than benzene or toluene when considering injury to nervous tissue and activity of brain cells, but is less than that of the longer chain alkylated benzenes.

Metabolism of xylene isomers by organisms results in oxidation to toluic acid and subsequent combination with the amino acid glycine to form toluric acids. Another mechanism for detoxification involves the hydroxylation of xylene to produce xylenols. These metabolites conjugate with proteins and sulfhydryl groups in the blood to produce glucuronides and some ester sulfates, which are easily excreted. The metabolites of xylene are much less toxic to mammalian systems than the biotransformation products of benzene, especially catechol (Laham, 1970).

Toxicity to Aquatic Organisms

o-Xylene in concentrations between 25 to 100 ppm exerted short-term toxicity to Chlorella vulgaris (Kauss et al., 1973). Recovery appeared to occur as a result of volatilization of this aromatic hydrocarbon, but at concentrations near its water solubility (171 ppm), algal growth was completely inhibited.

The acute toxicity of xylene to aquatic organisms varies considerably. Dowden and Bennett (1965) reported a median tolerance range of more than 100 ppm but less than 1,000 ppm for Daphnia magna exposed to xylene for 24 hours. Acute toxicity of the xylene isomers to fish is variable, with m-xylene typically the most toxic and o-xylene the least toxic. The TL_m values for xylene are slightly lower than those for benzene, indicating higher toxicity for these less water-soluble aromatics (Pickering and Henderson,1966). Pickering and Henderson determined that 50 percent survival over 96 hours occurred at 26.7 ppm for fathead minnows, 20.9 ppm for bluegill sunfish, 36.8 ppm for goldfish, and 34.7 ppm for guppies. These values compare favorably with previously reported toxic concentrations for short-term exposures to freshwater fishes (Shelford, 1917; Hubault, 1936). Applegate et al. (1957) discovered that rainbow trout and larval sea lampreys were not affected by m-xylene at concentrations of 5 ppm during a 24-hour exposure, but bluegills exhibited sublethal effects after 10-hr exposure. These tests (which were used to evaluate potential larvacides for control of sea lampreys) suggested differences in toxic susceptibility between coldwater and warm-water fishes.

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SECTION 7

TREATABILITY SCREENING TESTS

Screening tests were performed early in the project to determine whether the contract compounds were biodegradable and thus candidates for the biological countermeasure. At the same time, growth kinetics data were obtained if possible, to better define the methodology for the subsequent growth kinetics tests reported in Section 8. The procedures and results for the screening tests are described below.

PROCEDURES

Individual screening tests were made utilizing a two-liter sample in a batch, aerated plexiglass reactor 8.75 cm wide, 12 cm long, and 32 cm high. At the start of each test, each reactor was filled with 500 ml of sludge from a previous test or 500 ml of mixed liquor from a local sewage treatment plant. Two sources were available for mixed liquor--Friendswood, a combined industrial-municipal plant near Houston, and Govalle, a domestic waste treatment plant in Austin. At the start of this work, it seemed advisable to obtain sludge from sewage containing a heavy load of industrial wastewater. However, this precaution soon proved unnecessary.

The remaining volume of the reactor was filled with distilled water to which nitrogen, phosphorus, and an alkalizing agent, as well as the desired dosage of the test chemical, had been added. Initially, 150 mg/l glucose and 150 mg/l of glutamic acid were added to supplement the substrate for the microorganisms used. Later, however, acclimated sludge was used and glucose and glutamic acid were deleted. In one test, the glucose and glutamic acid were replaced by a 5% solution of yeast extract, which provided a source of vitamins, but did not add an additional carbon source.

The length of each test was normally 24 hours, although some tests were extended to 72 hours. Temperature was controlled at 24°C.

Suspended solids (total and volatile), oxygen consumption, pH, and total organic carbon (TOC) were monitored throughout the tests. Normally, samples were taken at frequent intervals over the 24-hour test period with the heaviest sampling density placed early in the test. When glucose and glutamic acid were added, a control reactor was used to subtract their contribution to the TOC measurments. Because glucose ($C_6H_{12}O_6$) has a molecular weight of 180.16, of which 40 percent is carbon, its initial contribution to the TOC measurements was 150 mg/l X 0.4 or 60 mg/l. Likewise,the glutamic acid ($C_5H_9O_4N$), with a molecular weight of 147.13 and a carbon content of 41 percent, contributed 62 mg carbon/l to the TOC measurements. Occassionally the TOC value for the control was greater than that for a chemical; such values were recorded as zero. A gas chromatograph was used to detect specific chemicals in a number of the treated effluents. Correlation between the gas chromatograph and the TOC results for phenols proved useful. Because of limitations of the instrument, however, the results with other chemicals proved less satisfactory.

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Wet mounts and gram stains were made from the contents of the reaction chambers to determine the types of microorganisms adapted to each chemical. Gram-negative and gram-positive bacilli, and gram-positive cocci were seen. Selection of a predominant organism was difficult with the gram stains, nevertheless, wet mounts did provide useful information.

RESULTS

The results of the screening tests are given below for each of the chemicals tested.

Aldrin

Four screening tests for aldrin using 9, 18, 27, and 36 mg/l aldrin as initial concentrations were performed on November 12-13, 14-15, 19-20, and 20-21, 1973. In none of the tests could aldrin be detected over and above the glucose and glutamic acid concentrations. It was concluded that aldrin either sorbed onto the reactor walls or sludge solids, or could not be detected by TOC or the flame ionization detector available on the gas chromatograph. Further tests to overcome these difficulties were to be performed as time permitted; however, none were continued.

Acetone Cyanohydrin

Acetone cyanohydrin ($(CH_3)_2C(OH)C \equiv N$, MW=85) contains a cyanide group and has toxic effects similar to those of acrylonitrile. The same enzyme system that detoxifies acrylonitrile (discussed below) also splits the analogous bond in cyanide ($-C \equiv N$) to ammonia and methane. This suggests that massive inoculation with Azotobacter or Clostridium, in any area when a spill of compounds that decompose to cyanide occurs, would be beneficial for mitigating the hazardous effects of the compound. The organisms used in these tests do not attack acetone cyanohydrin because the spatial arrangement of the two methyl groups prevents the enzyme from acting on the $C \equiv N$ groups. However, acetone cyanohydrin decomposes readily to form cyanide and thus is susceptible to the enzyme system under study.

Acrylonitrile

Acrylonitrile ($H_2C=CH-C \equiv N$, MW=53.06) is among the most dangerous of the high priority compounds investigated in this study. It is flammable at high concentrations, forms explosive mixtures with air, and even at low concentrations is highly toxic through the effect of the $-C \equiv N$ moiety (less than 1 gram being a potentially fatal dose for humans).

No bacteria were isolated that were capable of using acrylonitrile as a growth substrate. However, it was possible to detoxify acrylonitrile by use of an enzyme cross-reaction. The ordinary substrate of the enzyme nitrogenase is atmospheric nitrogen, N_2 or $N \equiv N$, which is reduced in certain microbes to two ammonia (NH_3) molecules that are readily assimilated into amino acids and proteins. Because of the similarity in bond structure between the natural substrate, $N \equiv N$, and the cyanide portion of acrylonitrile, $R-C \equiv N$, nitrogenase will also reduce acrylonitrile by the irreversible reacton illustrated below:

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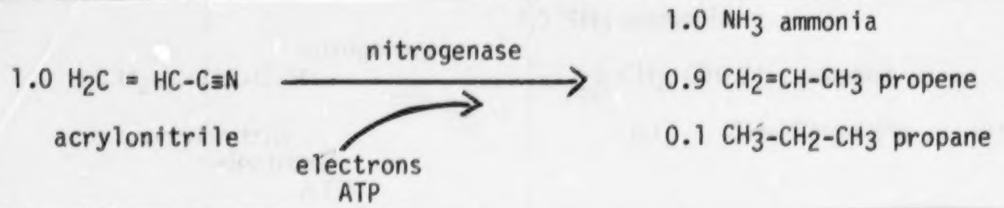

$$1.0\ H_2C = HC\text{-}C{\equiv}N\ \text{(acrylonitrile)} \xrightarrow[\text{electrons, ATP}]{\text{nitrogenase}} \begin{array}{l} 1.0\ NH_3\ \text{ammonia} \\ 0.9\ CH_2{=}CH\text{-}CH_3\ \text{propene} \\ 0.1\ CH_3\text{-}CH_2\text{-}CH_3\ \text{propane} \end{array}$$

Not only is the lethal cyanide group reduced in this reaction, but the ammonia produced is a beneficial nutrient. Propene and propane are inocuous compounds that are readily oxidized to CO_2 by a wide variety of soil bacteria.

Bacteria were available in the laboratory that were capable of producing the acrylonitrile-detoxifying enzyme in large amounts. The aerobic Azotobacter vinelandii and the anaerobic Clostridium pasteurianum are non-pathogenic soil saprophytes that may be conveniently stored for long periods then grown to large numbers, with high nitrogenase activity maintained. Although the enzyme can be purified and concentrated and these experiments were designed to illustrate the practical use of enzyme preparations to detoxify hazardous materials, whole cells can also be used. When properly prepared they maintain the enzyme in a stable condition over long periods. In addition, maintaining the enzyme in the bacteria permits the living cells to furnish the necessary metabolites to drive the reaction forward. With enzyme extracts it is necessary to add such auxiliary compounds as ATP, creatinine phosphate dithionite, and others, thus complicating the problem. Azotobacter and Clostridium cells are readily produced in massive quantities, the former being produced in the U.S.S.R. for many years as a soil inoculant and strains of the latter being cultured world-wide for industrial fermentations. Many strains and mutants of Azotobacter are maintained in the authors' laboratories and methods for readily securing additional mutants from this genus, which was formerly regarded as difficult to mutagenize, have been perfected.

Experiments in this laboratory showed the following:

1. It was necessary to exclude or at least reduce the natural enzyme substrate, nitrogen, if a significant rate of acrylonitrile reduction was to take place.

2 It was theoretically possible to reduce acrylonitrile at a rate equal to 20% of the nitrogen reduction rate.

3 Enzyme saturation by acrylonitrile was achieved at 50 mM (2.65 mg/l) in cell free systems, but in whole cell experiments optimum activity took place at about 1 mM (0.053mg/l) acrylonitrile - this difference was probably due to the toxicity of acrylonitrile to living cells.

4 Large-scale acrylonitrile reduction with cell-free enzyme preparations was not practical because: (a) the cell-free enzyme was extremely oxygen sensitive, (b) a large flow of high-energy electrons was required for activity, and (c) ATP was required in such large amounts (about 25 ATP's per acrylonitrile reduced) that adding it was impractical.

A useful method for the study of acrylonitrile degradation by nitrogenase-containing bacteria was developed. In this procedure, bacteria were grown to late log phase (about 16 hours) with ammonium in the medium, allowing rapid growth and a

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large crop. These bacteria were pelleted by centrifugation then resuspended in a nitrogen-free medium in a closed vessel having an air dispersion tube and a septum-stoppered sample port. The suspension of bacteria was then gassed continuously with non-nitrogen inert gas. Pure argon was used for anaerobes and a mixture of 80:20 (argon: oxygen) was necessary for aerobes. Maximum amounts of nitrogenase activity were present after approximately two hours under these conditions. When maximum nitrogenase activity was attained, 10 ml of the culture were dis-tributed by syringe to each of several 60-ml stoppered serum bottles containing a non-nitrogen atmosphere. Additions of acrylonitrile or other substances were then made and acrylonitrile decrease or propene and propane increase was determined gas chromatographically. The results of a typical experiment are shown in Figure 2. The Y-axis shows relative propene production after 120 minutes incubation at 33°C.

The 60-ml rubber-stoppered serum bottles contained an atmosphere of 80:20 (argon:oxygen), approximately 1×10^9 Azotobacter vinelandii cells in 10 ml of nitrogen-free growth medium, and the specified concentration of acrylonitrile. A control bottle containing A. vinelandii but no acrylonitrile, as well as a bottle containing 1.0 mM acrylonitrile in sterile growth medium, showed no detectable propene.

These experiments demonstrated an application of the use of microbial enzyme systems to detoxify compounds. The potential usefulness of this procedure, at the present time, was predicated on the premise that the spill could be covered with a plastic film after inoculation with the active bacteria. With further study it may be possible to secure strains that produce an enzyme with greater preference for the topic compound than for the natural substrate, but such study was outside the scope of this project.

The screening tests demonstrated that acrylonitrile could be decomposed biochemically, but the conditions for using this countermeasure in the field would be quite restrictive.

Benzene

Seven screening tests were conducted on benzene to determine the practicality of its removal under spill conditions. Previous work cited in the literature had shown benzene to be biodegradable only if presented to the organisms in the vapor phase. It was not known whether a more dilute solution could be decomposed than those used in the screening tests.

The screening tests of August 16-17 and 20-21, 1973 were carried out with the addition of glucose and glutamic acid to benzene concentrations of 400 and 800 mg/l. Detection of benzene through TOC measurement was not consistent because of masking by the glucose and glutamic acid. No benzene was detected on the gas chromatograph. Subsequent screening tests on November 1-2, 5-6, 7-8, and 19-20, 1973 using benzene at 100, 200, 400, and 800 mg/l without glucose and glutamic acid were carried out. In three of the four tests, methanol (500 mg/l) was added to keep the benzene in solution. In all these tests, benzene could not be detected on the TOC or the gas chromatograph instrument.

While it was known that benzene was highly volatile, the rate of volatilization from a reactor such as was used in these tests was thought to be low enough to be measured. Yang (1968), for example, measured air stripping rates for several chemicals and obtained the results shown in Table 4, assuming first order decay. For

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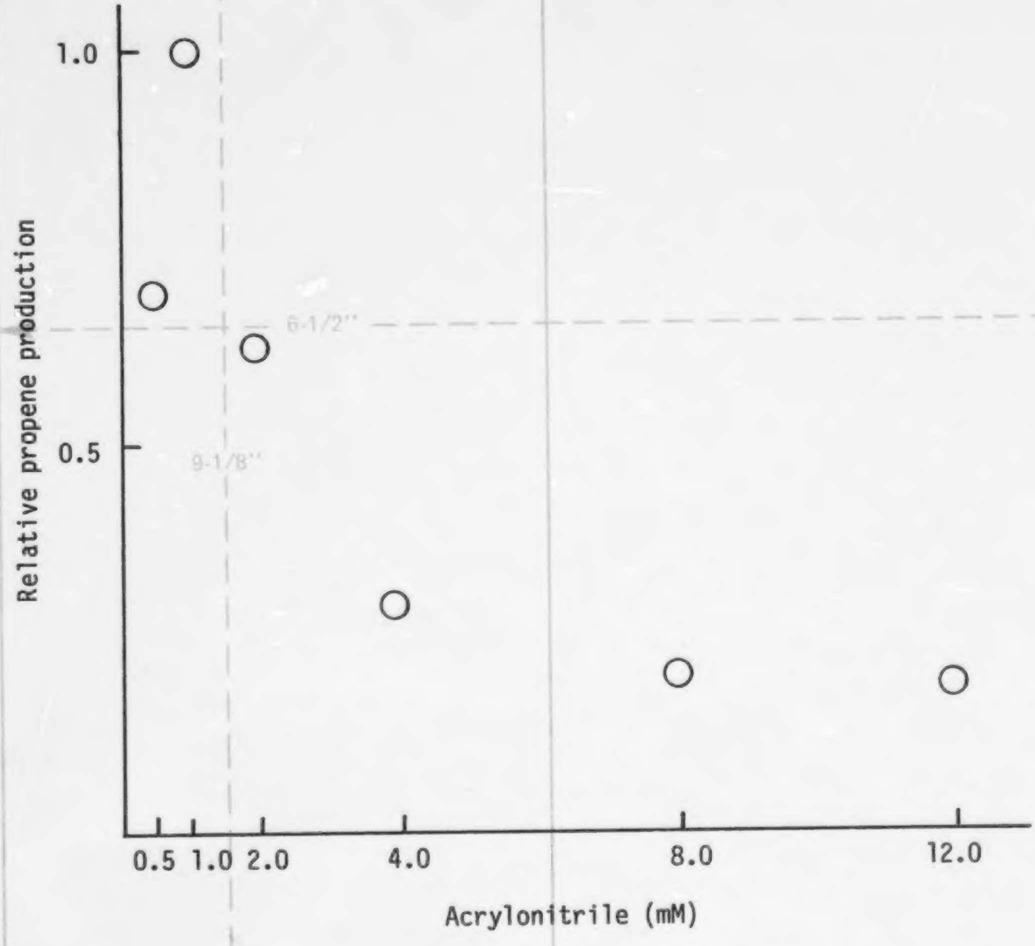

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Figure 2. Optimum acrylonitrile concentration for detoxification by whole cells of *Azotobacter vinelandii.*

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benzene, the rate of 1.71 d^{-1} would allow 18 mg/l of benzene to remain after one day when the initial concentration of benzene was 100mg/l. A final screening test was performed to determine the volatilization rate of benzene from a 200 ml volume contained in a 30 cm^2 surface area with several rates of stirring and aeration. An initial concentration of 1,000 mg/l was used.

TABLE 4. STRIPPING CONSTANTS FOR SELECTED CHEMICALS, ASSUMING $C=C_o \exp(Kt)^{-1}$

Material	$K(d^{-1})$	C(mg/1)
Nitrobenzene	0.843	250
Methanol	0.263	1,360
Ethanol	0.302	2,220
Refinery waste A	0.332	475
Refinery waste B	0.345	782
Benzene	1.71	100
Monochlorobenzene	0.969	100
Aniline	0.198	100

The results are shown in Table 5. It is apparent that with aeration, benzene will volatilize rapidly from the reactor and from sample vessels unless special precautions are taken.

It was concluded that the high volatility rate would restrict the use of a biological countermeasure for benzene. The engineering feasibility of covering a spill with an impermeable material and decomposing the spill material from the vapor phase was not evaluated, but is a possibility.

Table 5. Volatilization of Benzene in Stirred and Unstirred Containers

Mixing conditions	Removal rate mgB_r/d[1] $(mgB)\ (cm^2)$	 $T_{1/2}$[2] (hr)
Aerated	23.4	0.71
Stirred-large vortex	8.7	1.9
Stirred-small vortex	3.3	5.0
Stirred-no vortex	1.2	13.4
Unstirred	0.22	75.0

[1] Mg benezene removed (B_r) per hour per mg benezene (B) per cm^2 of surface area at 23°C.

[2] Time for 50% decrease in concentration at 23°C.

Isoprene

Four screening tests were performed with isoprene on December 11-12 and 12-13, 1973 and on November 2 and 17, 1974. The first two tests were designed to evaluate decomposition and the last two volatilization. Because of isoprene's low density (0.6810 at 20°C) and insolubility in water, it was not anticipated that the decomposition studies would be successful and they were not. The volatilization studies were qualitative in nature because of the tendency of isoprene to polymerize and resist measurement. Observations of the thickness of an isoprene layer on water over time and of the presence of isoprene by smell were used to show that, with aeration, isoprene vaporized within two hours after tests were initiated.

Methyl alcohol

Methyl alcohol (or methanol) was used in six screening tests on August 13-16 and 22-23, September 4-6, and October 29 to November 1, 1973, and on August 21-23 and 29-31, 1974. In the August 1973 tests, methanol plus glucose and glutamic acid were used. Initial methanol concentrations were 1,000, 2,000, 5,000, and 10,000 mg/l. The results showed that methanol was removed at an average rate of 0.51 d^{-1}, assuming first-order kinetics, or 0.0079 mg methanol removed per day per mg mixed liquor suspended solids (MLSS). It was assumed that the glucose and glutamic acid utilization did not interfere or compete with methanol decomposition.

A subsequent study was performed in September 1974 to determine whether glucose and glutamic acid were needed. The results showed that the first order removal rate for 5,000 mg/l methanol was 0.69 d^{-1} without glucose and glutamic acid, compared to 0.60 d^{-1} with glucose and glutamic acid. It was clear from this study that sludge from a local treatment plant could be used to decompose methanol with only mineral salts added.

Another screening test was designed to develop initial data on growth kinetics from batch tests. A 72-hour batch test was performed using initial concentrations of 1,000, 2,000, 5,000, and 10,000 mg/l methanol and activated sludge from the local Govalle Wastewater Treatment Plant. The sludge had been acclimated over a two-day period so that methanol was the sole carbon source. Using the calculation methods of Ford and Eckenfelder (1970), the removal rate, k, was found to be 0.005 mg methanol removed per day per mg MLSS. The microorganism yield was estimated to be 0.45 mg MLSS produced per mg methanol removed, while the decay rate, b, was found to be very close to zero.

The last two screening tests, carried out in August 1974, were designed to provide information for continuous-flow bioassays, which will be discussed in Section 8. These screening tests were "fill-and-draw" batch tests in which the reactor was filled with a mixture of activated sludge, mineral salts, water, and the desired concentration of substrate. After a specified period, a portion of the reactor contents (especially the MLSS) was removed and a fresh solution of test chemical,mineral salts, and water was added to bring the mineral salts and chemical concentrations back to their original levels. After operating for some time, the MLSS concentrations stabilized for the cell residence times produced in each reactor, as they would in a continuousflow reactor, and better estimates of removal efficiency, cell yield, and decay rates could be obtained. These tests yielded the desirable information for the continuous-flow tests, the results of which will be presented in Section 8.

At least five distinct cultures of bacteria were isolated on methanol agar from a methanol-adapted sludge. The cultures undoubtedly included the classical Methanomonas methanica (an organism that grows only on methane or methanol), as well as a Pseudomonas species, which has less restricted substrate capabilities.

Nitrophenol

Three chemical forms of nitrophenol were tested: o-nitrophenol (or 2-nitrophenol, melting point 44.9°C), m-nitrophenol (or 3-nitrophenol, melting point 97°C), and p-nitrophenol (or 4-nitrophenol, melting point 114°C). Each form is only slightly soluble in water and imparts a distinctly yellow color to the solution.

The initial screening on each of the three nitrophenol isomers was carried out on July 30-31, August 1-2, 2-3, 7-10, and 22-23, 1974. Twenty three tests were performed with glucose and glutamic acid. Separating the decomposition of nitrophenol from the glucose and glutamic acid was difficult, although there was evidence that decomposition of the nitrophenol was occurring. Subsequent screening tests on September 25-26, 26-27, and 27-28, 1973 were performed to elucidate the importance of the initial form of the nitrophenol -solid or liquid. Spills of nitrophenol in the solid form would require time for dissolution before decomposition could occur. Again, glucose and glutamic acid masked the results. The last four screening tests were conducted on nitrophenol on January 3-7 and 7-10, May 22-30 and June 11 to July 8, 1974. The first two of these tests used a pure culture of bacteria isolated by the microbiological group at the University of Texas. p-Nitrophenol concentration was determined by a correlation between concentration and light absorption (see Figure 3). It had been noted in earlier tests that the yellow color present in each reactor at the start of the tests would persist for some time then suddenly disappear. Disappearance of the color was considered to be tentative evidence of p-nitrophenol decomposition. The later of the two screening tests used acclimated activated sludge and followed the disappearance of p-nitrophenol by TOC measurements. An example of these results is

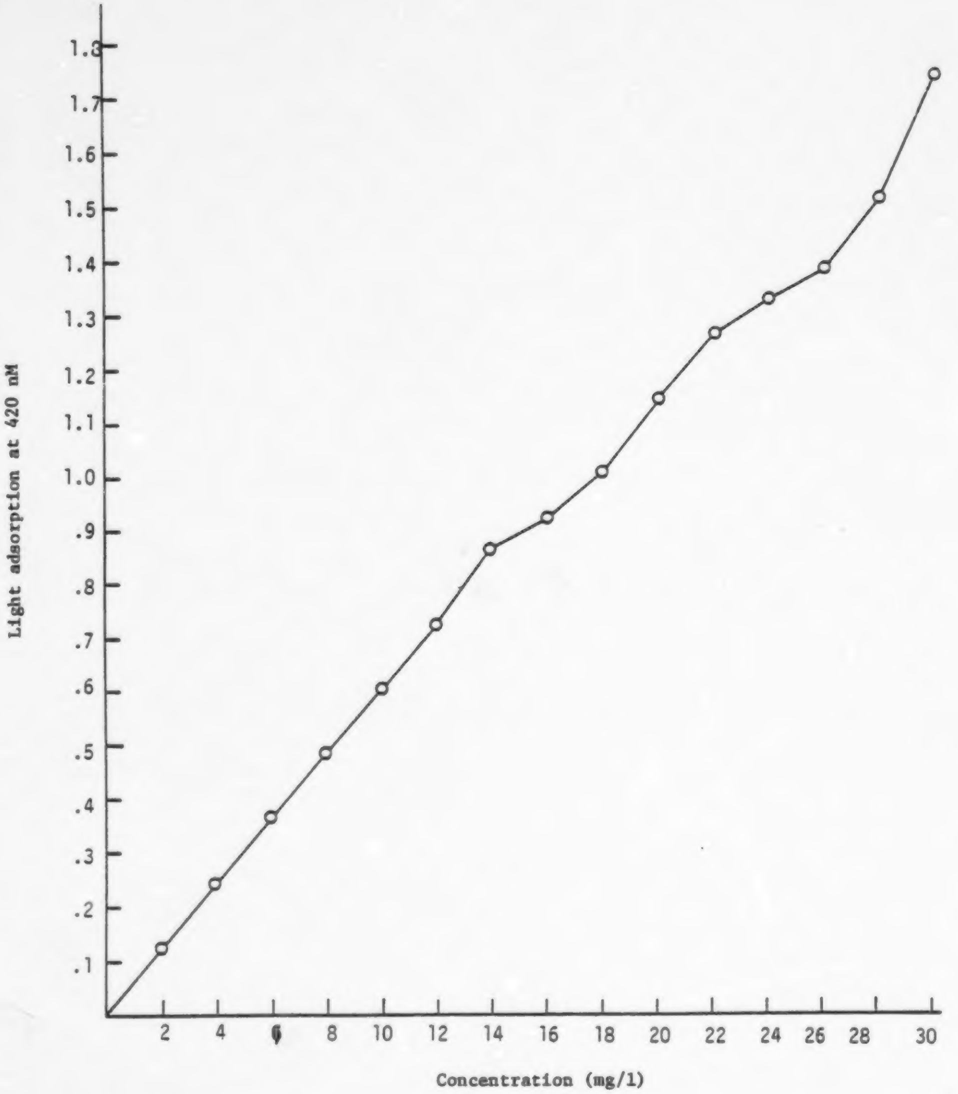

Figure 3. Relationship between p-nitrophenol concentration and light adsorption.

shown in Figure 4, in which the decrease in TOC after an initial 24- to 30-hour period, as well as the time at which the yellow color disappeared, is shown. Apparently, more time was required for the bacteria to attack the nitrophenol molecule than to attack the color producing group (probably the nitrogen group).

Further studies by the microbiology group showed that:

1 Two pure bacterial cultures could be isolated that decompose nitrophenol. From cytological evidence these appear to be a Micrococcus and a Pseudomonas. Both organisms decomposed p-nitrophenol in concentrations up to 160 ppm within 48 hours. Higher concentrations were attacked, but the time for decomposition was prolonged.

2. The optimum pH for bacterial activity was 6.8 to 7.6. Ammonia and mineral salts had to be added to the medium and at least 10 ppm glucose and 10 ppm yeast extract were needed.

3. The carbon in p-nitrophenol was released as CO_2. This was demonstrated by synthesizing p-nitrophenol uniformly labeled with radioactive carbon and by trapping the CO_2 that resulted from the bacterial decomposition of the radioactive compound. Measurement of the radioactivity showed that the carbon in p-nitrophenol was converted almost quantitatively to CO_2.

4. The fate of the nitro group on the p-nitrophenol was not determined, but it was not used to satisfy the nitrogen requirement of the organism. Other workers have shown that when pure cultures are used, the nitrogen appears in the medium as nitrite.

5. Initial studies indicated that the nitro group is cleaved from the aromatic ring and is not immediately reduced. The phenol that remains is oxidized to catechol by some cultures.

Nonyl phenol

Because of the chemical nature of nonyl phenol (water insoluble, adhesive), it was difficult to perform screening tests in batch reactors. The three tests performed on August 7-8, 9-10, and 22-23, 1973 did not yield conclusive evidence of decomposition. However, the microbiology group demonstrated that nonyl phenol was readily degraded by bacteria that line up on the medium nonyl phenol interface, disperse the nonyl phenol in smaller droplets, and eventually consume it. A mixed population was observed, but pure cultures were not isolated.

Phenol

Anticipating that phenol might be used to demonstrate spill control through biological countermeasures in pilot-scale studies later in the project, a substantial number of screening tests were performed.

The earliest screening tests on phenol were carried out on July 10-18, 23-24, 24-25, 25-26, and 26-27, 1973 using concentrations of 600 and 800 mg/l phenol (460 and 626 mg/l as TOC). The results, shown in Figures 5 and 6, show that over 90 percent removal was obtained in every case within 24 hours. Subsequent tests on August 16-17 and 22-26, 1973 were designed to show the importance of acclimation and the ability of pure cultures of phenol-decomposing microorganisms to degrade phenol. The pure

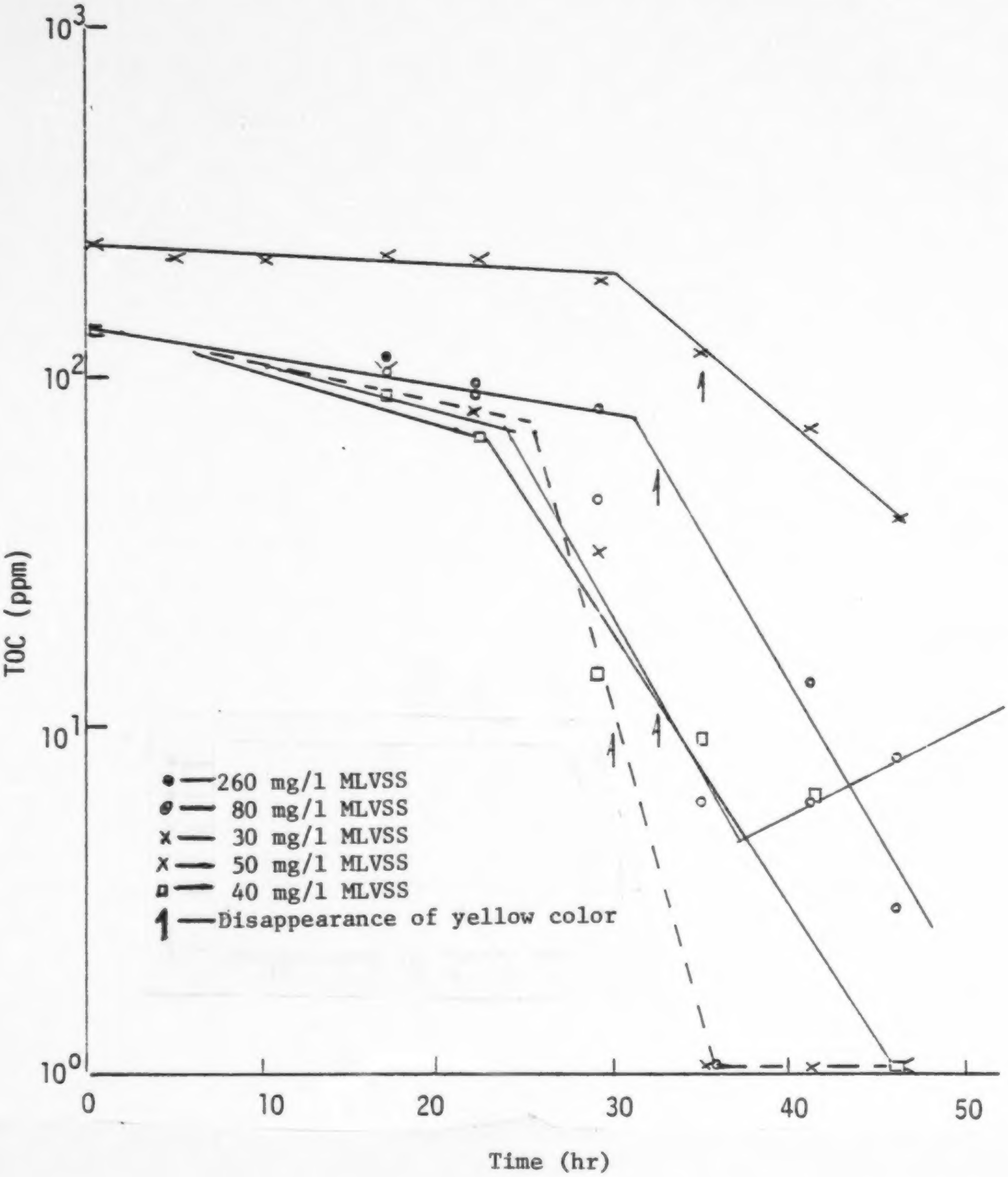

Figure 4. p-Nitrophenol intensive sampling - July 8-10, 1974.

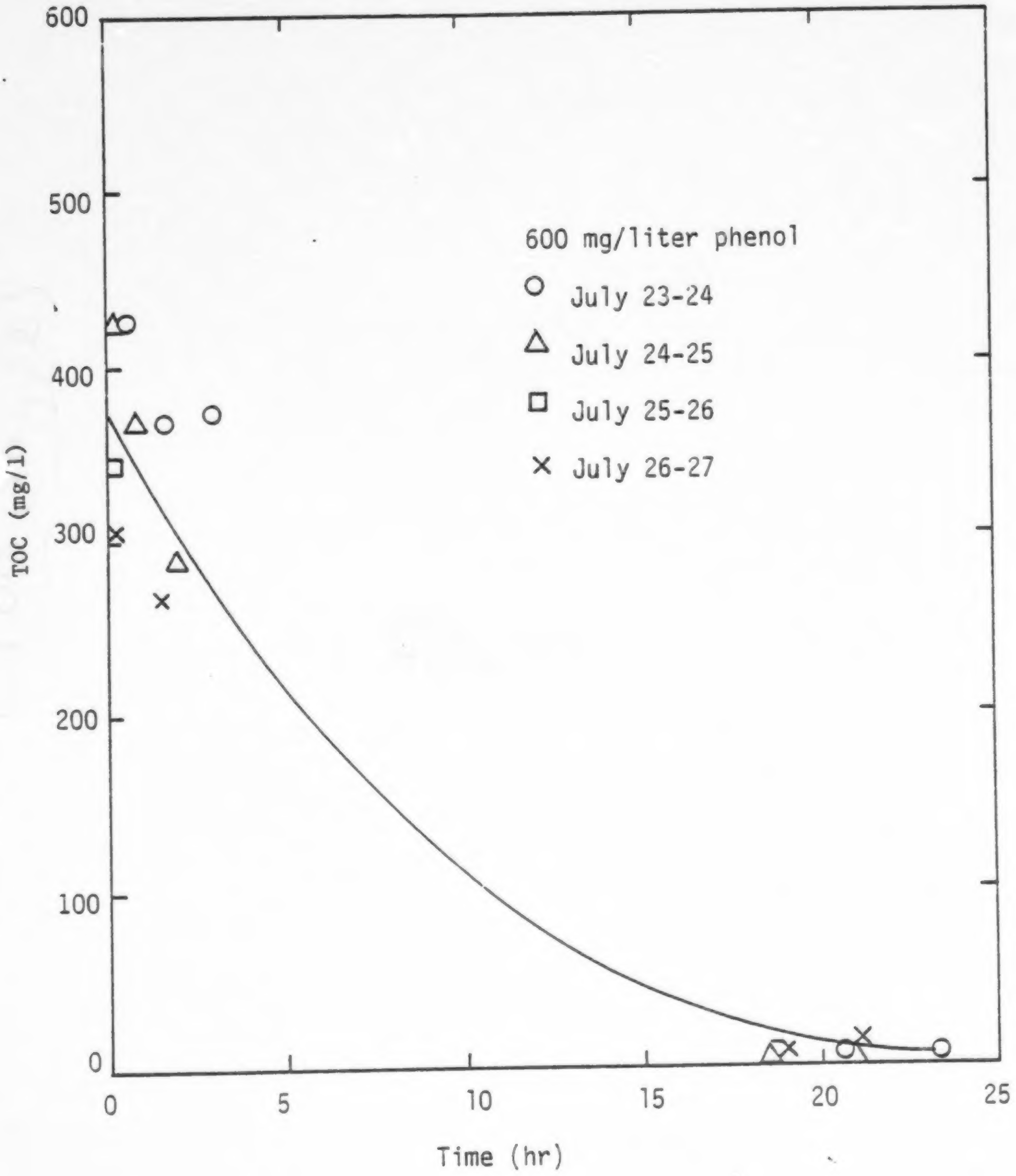

Figure 5. Degradation of phenol measured as total organic carbon.

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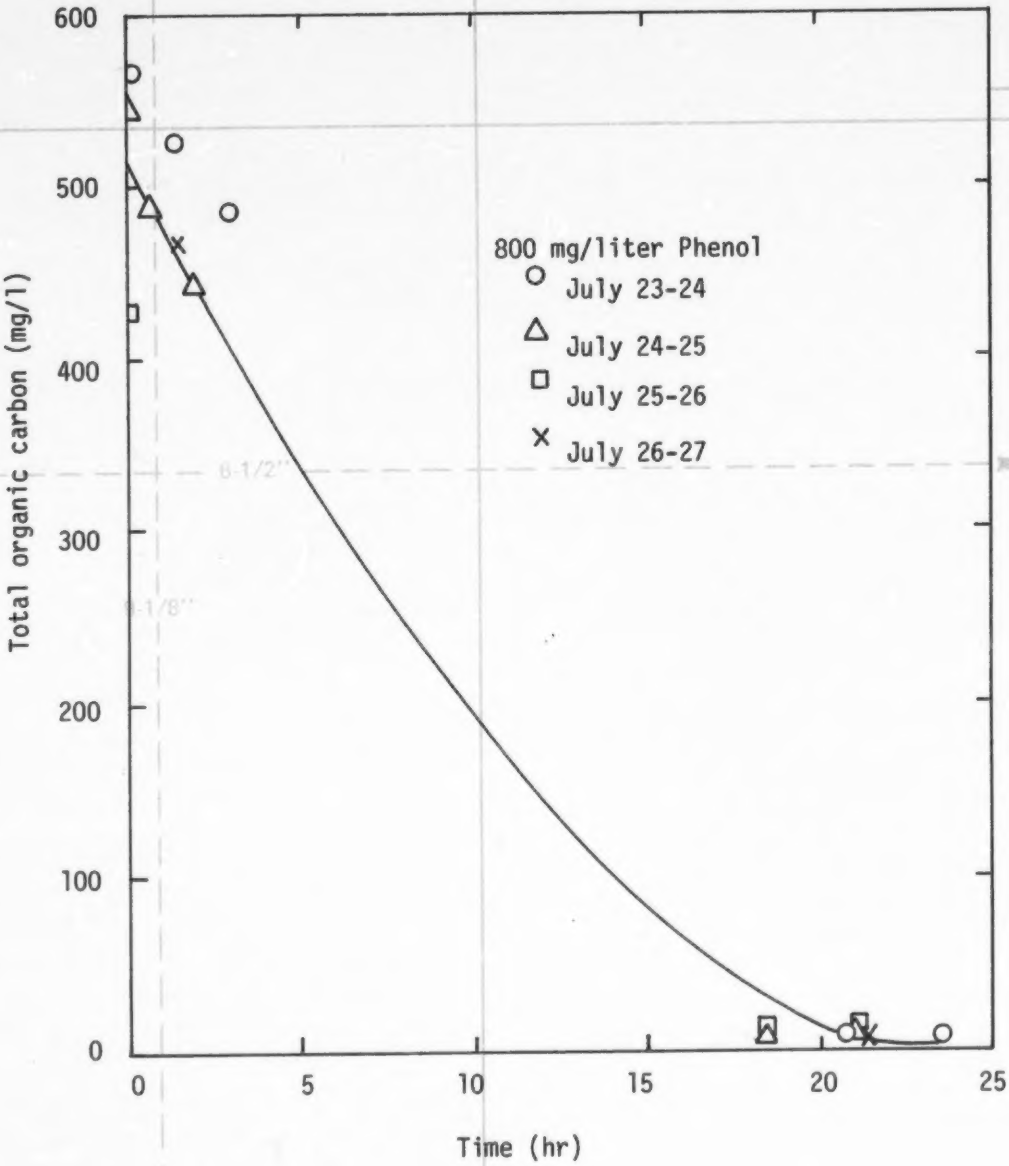

Figure 6. Degradation of phenol measured as total organic carbon.

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cultures of bacteria and yeastlike materials had been developed by the microbiology group of the University of Texas.

The results of the later tests are depicted in Figures 7 and 8. Both of the tests showed that an activated sludge developed in the laboratory consistently gave the best results. The experiment depicted in Figure 7, which like the previous runs utilized glucose and glutamic acid, showed that these two nutrients apparently did not make a suitable source of carbon for the yeast-like material provided by the microbiology group. The test depicted in Figure 8 was different in two respects. First, it was run for three days instead of one day. Secondly, the glucose and glutamic acid were withheld and the chambers with the yeast extract were closed . Under these circumstances the yeast culture, the bacterial culture, and the un-treated sludge took approximately three days to decompose the phenol. The treated sludge in the presence of glucose and glutamic acid reduced the phenol in five hours, but it took approximately 24 hours to accomplish the same removal in the absence of glucose and glutamic acid. The extremely low value for Figure 8 was a result of the high suspended solids level (greater than 2,000 mg/l).

Subsequent screening tests were carried out to : (1) show the importance of supplemental nutrients (glucose and glutamic acid) (August 28-30, 1973); supplemental nutrients apparently did not aid removal, (2) evaluate the ability of bacteria to attack high phenol concentrations (September 4-6, 1973); greater than 94% phenol removal was observed at initial concentrations of 900, 1,200, 1,500, and 2,000 mg/l in one day, (3) obtain a more precise determination of the effects of additional nutrients (September 18-19, 19-20, and 20-21, 1973), and (4) determine the effects of microorganism inoculum size (January 15-16 and 22-27 and March 5-6 and 21-22, 1974); increasing the inoculum by decreasing the phenol mass to microorganism mass ratio increased the rate of phenol removal as shown in Figure 9. These studies provided valuable guidance for the more detailed studies subsequently carried out.

Organisms identified in the cultures used to decompose phenol were generally of the genus Pseudomonas.

Styrene

Screening tests were performed on August 20-21 and 21-22, 1973 using concentrations ranging from 100 to 1,000 mg/l of styrene. The results of these tests indicated that little, if any, decomposition of styrene occurred. Later tests on October 2, 1974 showed that styrene volatilized within a few hours and, like benzene and isoprene, may not be a suitable candidate for the biological countermeasure.

Toxaphene

While a number of tests were performed on toxaphene on November 5-6, 7-8, 12-13, 19-20, and 20-21, 1973, gas chromotographic analysis of samples taken from the reactors could not detect toxaphene. Through TOC analysis, it was not possible to detect decomposition above the level due to addition of glucose and glutamic acid. Apparently, the toxaphene was adsorbed onto the activated sludge solids and did not appear in the filtered samples that were analyzed. These screening tests were generally inconclusive regarding toxaphene decomposition.

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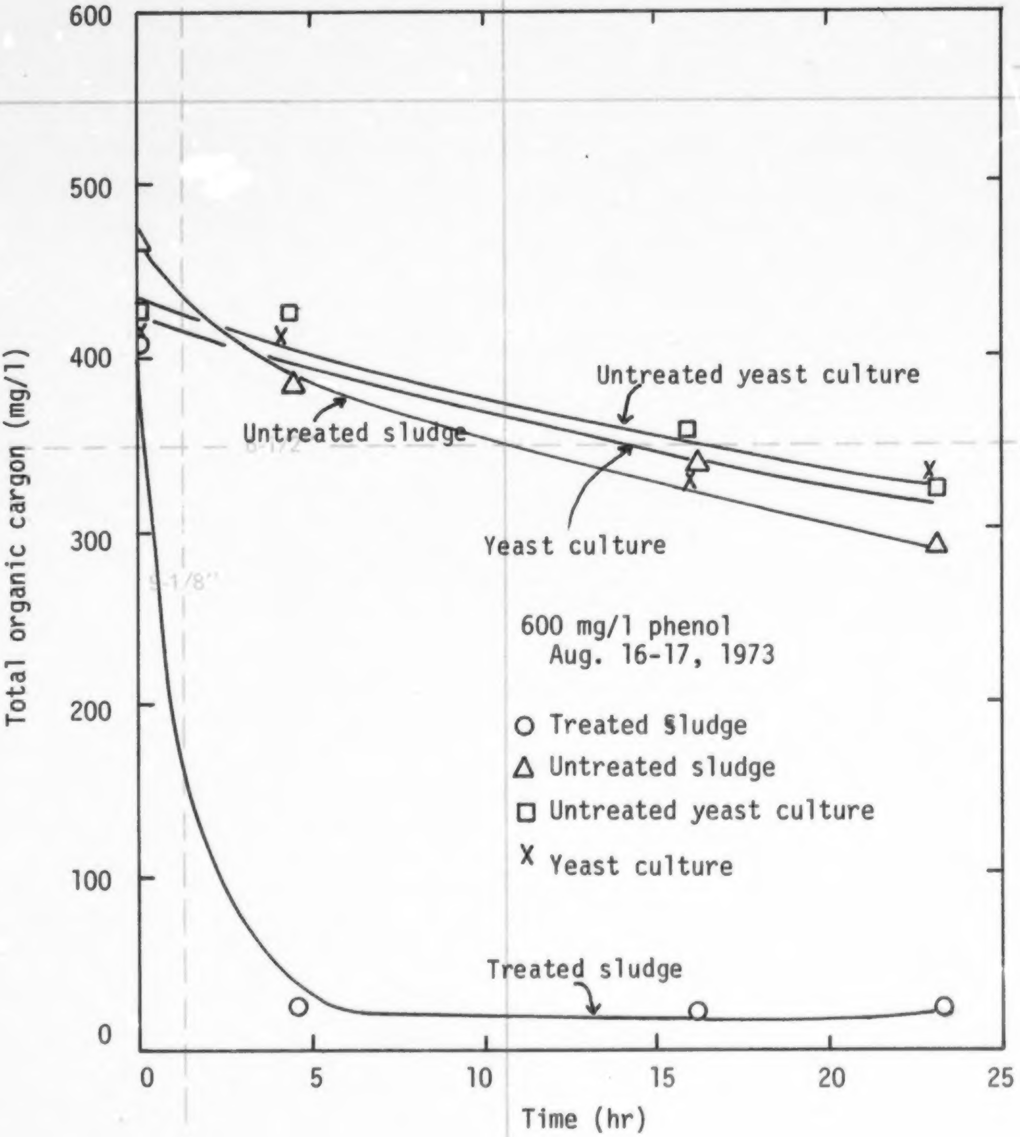

Figure 7. Degradation of phenol using various inoculums.

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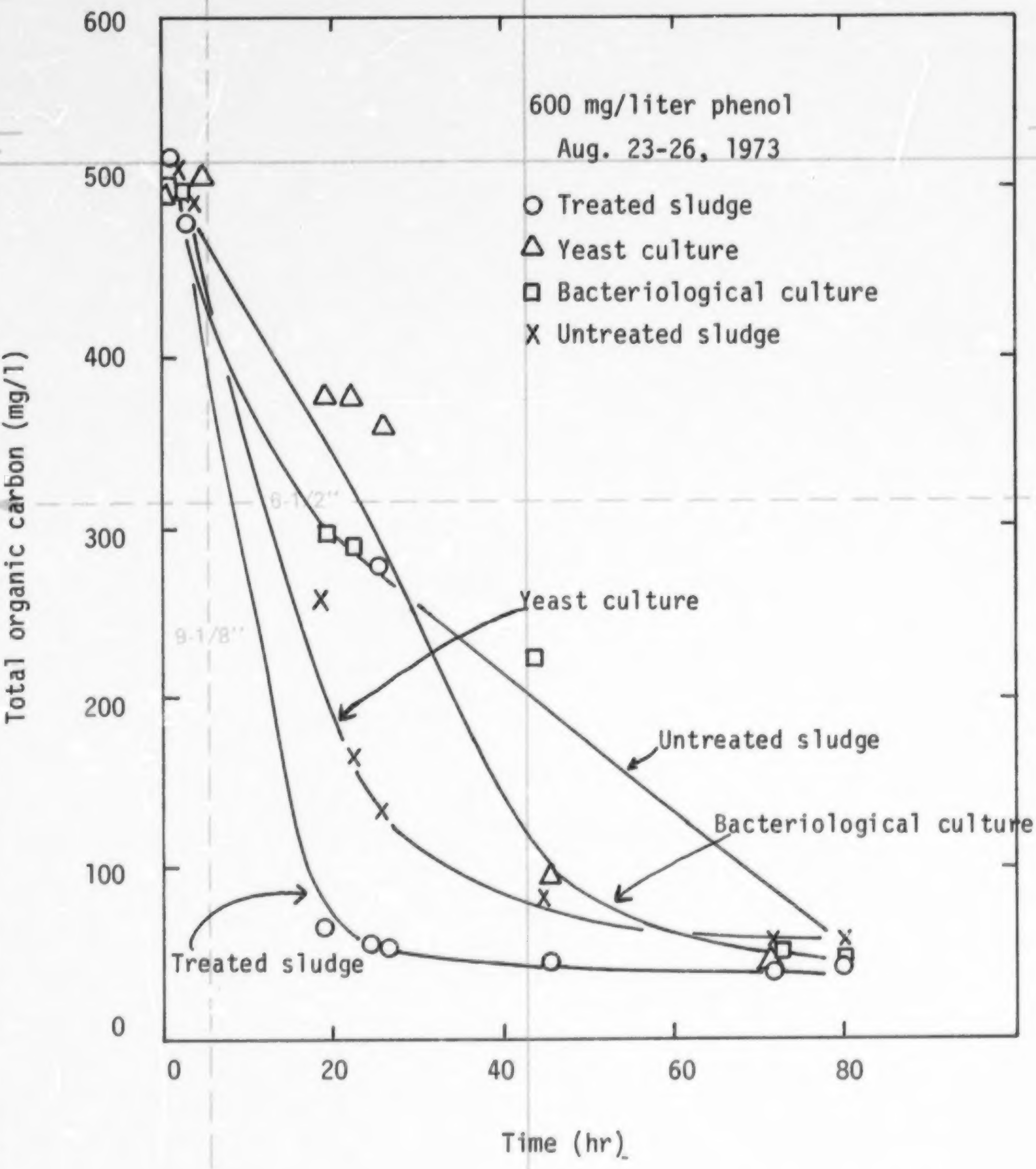

Figure 8. Degradation of phenol using various inoculums.

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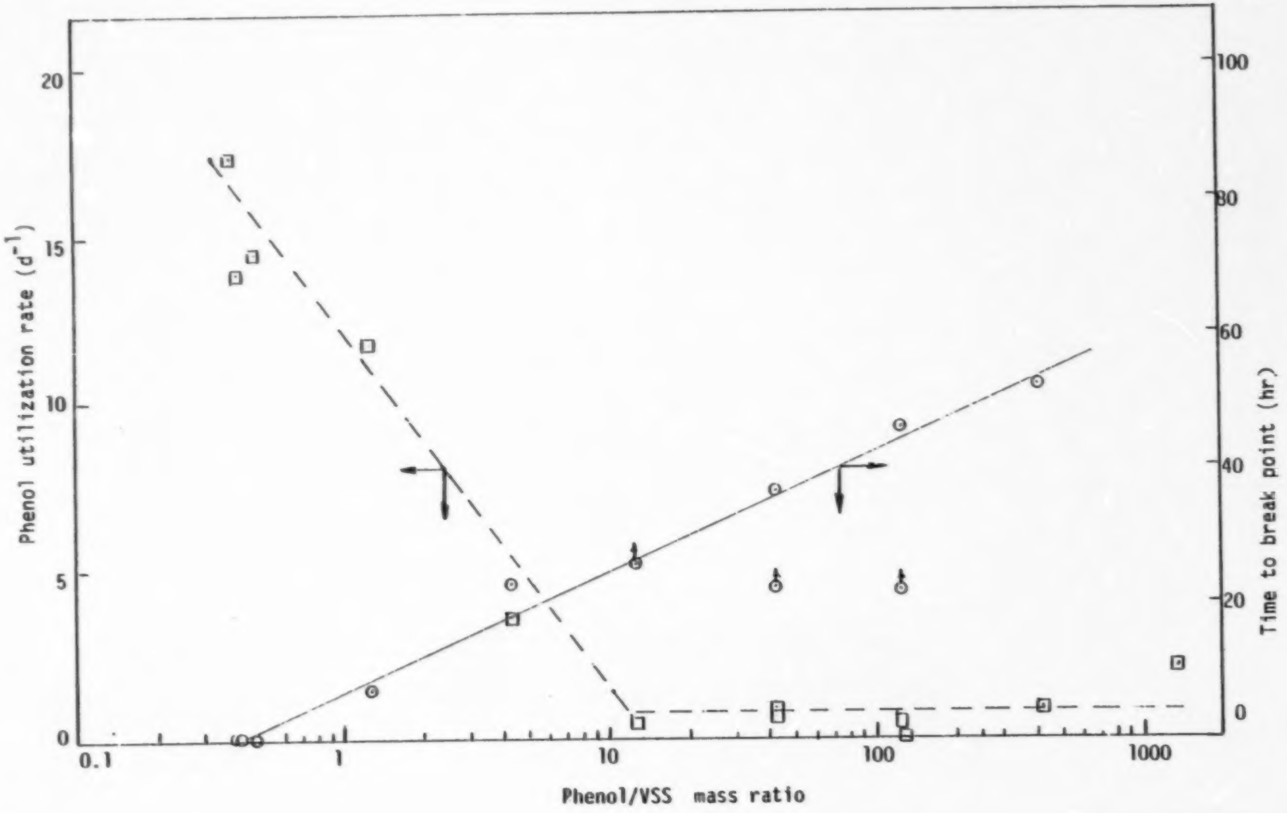

Figure 9. Effect of phenol/inoculum mass ratio on phenol removal.

Xylene

Results of the seven screening tests conducted on ortho-, meta-, and para-xylene on July 18-20, 23-24, 24-25, and 25-26, and on September 13-14, 17-18, and 18-19, 1973 indicated that xylene volatilized rapidly. As with other chemicals tested, the results for the early screening tests for xylene were confused because of the added glucose and glutamic acid. The later tests, which confirmed volatilization, also showed that xylene may not be amenable to the biological countermeasure unless special precautions can be taken to keep it in solution.

Summary

At the onset of studying this series of chemicals, it was decided that their behavior would be sought as a contaminant in domestic sewage. Accordingly, their behavior with time was defined in terms of the TOC content of a volume of liquid in the batch aerator. Of the 11 different compounds studied, phenol, methyl alcohol, and nitrophenol responded best in terms of removal. It was also shown that acrylonitrile, acetone cyanohydrin, and nonyl phenol could be decomposed. Isoprene, benzene, styrene, and the three isomers of xylene, because of their volatility, may not be candidates for biological countermeasures unless the chemical can be contained in the gaseous phase or kept in solution. Aldrin and toxaphene could not be shown to be decomposable in these tests. Of the contract list of chemicals, those not tested were the chemicals listed as groups of chemicals, for example, cyclic insecticides.

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SECTION 8

BIOLOGICAL COUNTERMEASURE TREATMENT SYSTEM

Following the screening tests, the three chemicals that appeared to be most amenable to biological countermeasures (phenol, methanol, and p-nitrophenol) were subjected to more intense studies designed to delineate growth kinetic coefficients, the effects of environmental variables, and other information necessary to conduct pilot scale countermeasure tests. The methods used and the results obtained are described in this section.

SUBSTRATE REMOVAL AND BACTERIAL GROWTH KINETICS

The substrate removal rate by microorganisms is frequently approximated by the following expression (Metcalf and Eddy) 1972, which is similar to the one developed by Monod (1949) to describe the relationship between the concentration of a limiting nutrient and the concentration of enzyme; that is:

$$\frac{dS}{dt} = - \frac{kSX}{K_s+S} \qquad (14)$$

where: S = concentration of substrate (M/L^3),

t = time (T)

k = substrate utilization rate coefficient ($1/T$),

X = concentration of microbial mass (M/L^3), and

K_s= substrate concentration at which the substrate removal rate is one half of the maximum rate (Michaelis-Menten constant) (M/L^3).

McCarty (1964) and Servizi and Bogan (1963) pointed out that the ratio of the cell mass produced to the free energy released by the oxidation of substance was almost constant and that chemical oxygen demand (COD) or biochemical oxygen demand (BOD) had a rough linear relationship with free energy. The following approximate relationship between bacterial growth and substrate utilization is commonly used (Stanier et al., 1970; Heukelekian et al., 1951):

$$\frac{dX}{dt} = - a \frac{dS}{dt} - k_d X \qquad (15)$$

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where: a = growth yield coefficient (biomass produced (M/L^3)/substrate utilized (M/L^3) and
k_d = cell decay coefficient (1/T).

Factors Affecting Microbial Activity

Temperature--

The modified Arrhenius equation is widely used to describe the temperature effect on the substrate removal rate (Eckenfelder, 1967; Carpenter et al., 1968); that is:

$$k_{T2} = k_{T1}\,\theta^{(T2-T1)} \qquad (16)$$

where: $k_{T1,T2}$ = substrate removal rate coefficient at temperature T1 or T2°C and θ = temperature coefficient.

Reported values for the temperature coefficient, θ, vary widely starting from 1.0 (Eckenfelder, 1967; Carpenter et al., 1968; Wuhrmann, 1955; Eckenfelder and O'Connor, 1961; Howland, 1958; Schroepfer et al., 1960; Eckenfelder, 1966; Phelps, 1944; Zanoni, 1969; Novak, 1974), depending on the substance, temperature range, type of treatment facility, and the procedure for evaluation of the reaction rate coefficient. The variation of θ with temperature and substance concentration raised questions about the validity of Equation 16 for representing the effect of temperature on microbial activity (Zanoni, 1969; Novak, 1974).

pH and Salinity --

The pH of the internal environment of all living cells is believed to be approximately neutral and most organisms cannot tolerate pH levels below 1.0 or above 9.5. At low or high pH, acids (which tend to exist in undissociated forms) can penetrate into cells more easily because electrostatic forces cannot prevent them from entering. The permeated substances can upset the internal pH balance. As pH deviates from neutral, bacterial activity decreases (Metcalf and Eddy, 1972; Stanier et al., 1970; McKinney 1962; Randall et al., 1972).

Bacterial cells maintain an internal osmotic pressure equal to about a 0.85% solution of NaCl. When the environment has a lower osmotic pressure than the cell (hypotonic), water tends to permeate into the cell. Higher extracellular osmotic pressures (hypertonic) cause the contraction of the protoplasm as a result of water loss through the semi-permeable cell wall. A hypotonic environment (fresh water) is the normal condition for most bacteria and they tend to exist in a distended form, maintaining their shape within the cell wall. Bacteria can grow in media with salt concentrations ranging from less than 0.1% to about 10%, but their activities are impaired with increasing salinity (Stainer et al., 1970; Burnett, 1975).

Nutrients --

When abundant nutrients are supplied, bacterial growth results in a constant chemical composition. Bacteria consist of approximately 80% water and 20% dry matter, the latter being approximately 90% organic and 10% inorganic. The approximate formulation of the organic fraction is $C_5H_7NO_2$, while the inorganic fraction is

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approximately: 50% P_2O_5, 6% K_2O, 11% Na_2O, 8% MgO, 9% CaO, 15% SO_3, and 1% Fe_2O_3. It is generally agreed that nutritionally balanced wastes result in unrestricted bacterial growth and rapid decomposition of pollutants.

Helmers et al., (1951, 1952) reported that for successful removal of waste by the activated sludge process, nitrogen and phosphorus must be supplied at the proportion of $BOD_5 : N : P = 100 : 5 : 1$. Sawyer (1955) observed that the nitrogen requirement decreased with increasing biomass as a result of decreased cell production.

Wilkinson (1958) studied the influence of nitrogen, phosphorus, and sulfur on the production of polysaccharides by *Klebsiella aerogenes*. When the concentrations of these nutrients were lowered until they became growth limiting, the amount of polysaccharides produced per cell increased to a maximum level. At this point, the polysaccharide to nitrogen, phosphorus, and sulfur ratios were about 32, 40, and 17, respectively.

Symons and McKinney (1958) reported that the conventional activated sludge system functioned satisfactorily even in the absence of nitrogen for three to four weeks. More solids were produced in the absence of nitrogen and the solids produced were high in polysaccharides. The authors concluded that partial satisfaction of the nitrogen requirement could result in a stable system.

Gaudy and Engelbrecht (1960, 1961) reported that regardless of the presence of extracellular nitrogen, the organic load was removed at the same rate suggesting the possibility of an amino acid pool within the bacterial cell.

Despite the fact that trace elements are required in minute amounts, they exhibit a pronounced effect on bacterial activity. Mg^{++}, Mn^{++}, Fe^{++}, Ca^{++}, etc., act as cofactors for respiratory processes (Stanier et al., 1970; Oginsky and Umbreit, 1954).

Oxygen Requirement --

In the aerobic process of organic substance stabilization, the molecular form of oxygen is the only final hydrogen acceptor. Thus, oxygen demand is a direct function of biological metabolism and the oxygen requirement is directly related to the amount of organic matter decomposed and rate of endogenous respiration. The oxygen utilization rate can be formulated as follows (Eckenfelder and O'Connor, 1961):

$$Rr = - a' \frac{dS}{dt} + b' X, \qquad (17)$$

where: Rr = oxygen utilization rate ((mg/l O_2)/time),
a' = oxygen required per substrate utilized, and
b' = oxygen required per biomass for endogeneous respiration ((mg/l O_2)/(mg/l biomass)/time).

EXPERIMENTAL METHODS

Equipment and Reagents

The analytical instruments used for these tests included an Expanded-Scale pH Meter from Beckman, a Salinity Refractometer (automatic temperature-compensated) from American Optical Co., a Galvanic Cell Oxygen Analyzer from Precision

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Scientific, a 15-liter-per-minute capacity air flow meter from Gelman Instrument Co., and a Beckman Model 915 Total Organic Carbon Analyzer. Aerated batch reactors made of plexiglass units 8.75 cm wide, 12 cm long, and 32 cm high were graduated at 25-ml intervals so that water loss by evaporation could be easily compensated. The reactor volume was either 2 or 3 liters.

The basic substrates used were liquified phenol, methanol, and glucose.

Nutrient Solution I was prepared as a nitrogen and phosphorus source and as a pH buffer for phenol- and methanol-acclimated activated sludge and consisted of: 320 g/l K_2HPO_4, 160 g/l KH_2PO_4, and 120 g/l NH_4Cl.

Nutrient Solution II was prepared as a nitrogen and phosphorus source for phenol-acclimated activated sludge and consisted of: 120 g/l NH_4Cl and 29 g/l KH_2PO_4.

Nutrient Solution III was prepared as a nitrogen and phosphorus source for methanol-acclimated activated sludge and consisted of: 91 g/l NH_4Cl and 21 g/l KH_2PO_4.

A mineral solution to supply trace elements for phenol- and methanol-acclimated activated sludge was made of: 15 g/l $MgSO_4.7H_20$, 0.5 g/l $FeS0_4.7H_20$, 0.5 g/l $ZnS0_4.7H_20$, 0.4 g/l $MnSO_4.H_20$, and 2 g/l $CaCl_2$.

A silver nitrate solution (1,000 mg/l as Ag) was used as an enzyme inhibitor.

For pH adjustment, $NaHCO_3$ solution (100 g/l), hydrochloric acid (1 N), or sodium hydroxide solution (1 and 6 N) was added and for salinity adjustment, "Synthetic Sea Salts" from Aquarium System, Inc., Eastlake, Ohio, was used.

For the determination of hardness, a buffer solution of 4.716 g/l disodium salt of EDTA, 3.12 g/l $MgSO_4.7H_20$, 67.6 g/l NH_4Cl, and 572 ml/l NH_40H (conc.) was made. The inhibitor was 50 g/l $NaS.9H_20$, the dry powder indicator was 0.5 g Eriochrome Black T and 100 g NaCl, and the titrant was a 0.01 M EDTA solution.

All chemicals used were reagent grade.

Acclimation and Feeding of Activated Sludge

Mixed liquor from the Govalle domestic wastewater treatment plant in Austin, Texas was used as seed. Supernatant from the mixed liquor was decanted after sludge settlement, and substrate and nutrients were fed to the sludge. Tap water was used as dilution water. After one to three days of aeration, ten percent of the total mixed liquor was wasted. The above procedure was repeated throughout the test period. Ten ml each of Nutrient Solution I and the mineral solution were fed each time. The substrate feeding schedule for phenol acclimated sludge was:

first day ....phenol 0.1 ml/l, glucose 940 mg/l
second day ....phenol 0.4 ml/l, glucose 750 mg/l
third day ....phenol 0.8 ml/l, glucose 500 mg/l
fourth day ....phenol 1.2 ml/l, glucose 250 mg/l
fifth day to
completion ....phenol 1.6 ml/l (1,200 mg/l TOC).

The feeding schedule for methanol-acclimated sludge was:

first day ....methanol 2.0 ml/l, glucose 940 mg/l
second day ....methanol 3.5 ml/l, glucose 750 mg/l
fourth day ....methanol 5.0 ml/l, glucose 500 mg/l
seventh day to
completion ....methanol 7.0 ml/l (2,000 mg/l TOC).

After the initial acclimation stage, phenol sludge was fed every day and methanol sludge was fed once every three days. The sludge was kept in the temperature range from 17° to 21°C.

The biological treatability and countermeasure application tests were done after at least three weeks of acclimation, when the sludge production reached a constant level. Sludge production was checked by measuring total suspended solids (TSS) and volatile suspended solids (VSS) before and after feeding the sludge. At steady state, the ratio of VSS to TSS ranged from 85% to 92%.

Substrate Removal and Bacterial Growth Kinetic Study

Substrate removal and bacterial growth kinetics tests were conducted at temperatures of 5°, 20°, and 28°C. For the tests at 5° and 28°C, the sludge cultures were acclimated to the new temperatures by placing the sludge at the respective temperature until 1,200 mg/l of phenol or 2,000 mg/l of methanol, as TOC, was completely removed. One day was adequate for the phenol sludge to remove the substrate. For the methanol sludge, three days were required to decompose the substrate completely at 28°C, while one week was required at 5°C.

The acclimated sludge cultures were elutriated with distilled water several times to reduce the residual organic carbon contents. The washed sludge was transferred to 2-liter reactors and fed with the phenol or methanol substrate and 10 ml/l each of Nutrient Solution I and mineral solution. The reactors were filled with distilled water up to the 2-liter mark and aerated.

The initial concentrations of VSS and TOC for the phenol study ranged from 300 to 4,000 mg/l and from 160 to 800 mg/l, respectively, and those for the methanol study ranged from 200 to 1,600 mg/l and from 500 to 1,000 mg/l, respectively. To calibrate the methanol loss by stripping, one reactor was not supplied with sludge in methanol tests at each temperature. Twenty-five ml of mixed liquor samples were withdrawn at certain time intervals after aeration for the determination of MLVSS and filtrate TOC. One drop of silver nitrate solution was added to each of the samples during filtration to inhibit further enzymatic activity and to reduce time measurement error caused by duration of filtration. For the determination of TOC, total carbon and inorganic carbon were analyzed. The residual TOC contributed by the mixed liquor, excluding substrate, was measured and subtracted from the sample TOC. The reactors were graduated and water loss by evaporation was made up with distilled water. pH and temperature were measured throughout the reaction periods. All other procedures were performed according to Standard Methods (APHA et al., 1970).

pH and Salinity Effects Study

Tests to evaluate the effects of pH and salinity on phenol and methanol removal were conducted at all combinations of temperature (5°, 20°, and 28°C), pH (5,6,7,8, and 9), and salinity (0, 10, and 35 ppt). Two additional salinities (5 and 20 ppt) were tested at 5°C to determine their effects on phenol removal. Salinity measurements refer to the dilution water salinities.

The activated sludge was prepared in the same manner described in the previous section and was acclimated to each test temperature but not to the various pH levels and salinities. The phenol sludge was fed with 5 ml/l of Nutrient Solution II, ten ml/l of mineral solution, and 720 mg/l of phenol as TOC. The methanol sludge was fed with 10 ml/l each of Nutrient Solution III and mineral solution, and 1,050 mg/l of methanol as TOC. The initial MLVSS ranged from 1,000 to 1,500 mg/l and from 450 to 700 mg/l for phenol and methanol studies, respectively. At a given temperature, the initial MLVSS was equalized for all pH and salinity conditions. All sampling and analytical procedures were the same as described in the previous section. Temperature and pH were measured and pH was adjusted frequently throughout the reaction periods.

Nutrient Studies

Nutrient studies were conducted at room temperature, which was about 20°C. The nutrient requirements were estimated in the following manner.

From the kinetic study tests, it was concluded that approximately 65% of the substrate utilized resulted in cell synthesis. The carbon to nitrogen to phosphorus ratio of bacterial cells is approximately 100 : 23 : 4.6 (Novak, 1974). Thus, 720 mg/l of phenol as TOC feed will require 108 mg/l (720 x 0.65 x 0.23) of nitrogen and 22 mg/l (720 x 0.65 x 0.046) of phosphorus. In order to prevent nitrogen and phosphorus from becoming growth limiting, a 50% excess supply (162 mg/l of nitrogen and 33 mg/l of phosphorus) was considered to be adequate in the phenol tests. Similarly, 236 mg/l of nitrogen and 47 mg/l of phosphorus were adequate for 1,050 mg/l of methanol as TOC. The effects of limiting nutrients were studied using concentrations of nitrogen and phosphorus equal to, one-half, or one-fourth of the concentrations determined above, as well as a zero concentration. Other conditions included tests with and without minerals and tests with and without pH adjustment to the neutral range. For mineral supply studies, ten ml/l of the mineral solution was fed to the sludge. pH was adjusted using bicarbonate.

To observe substrate removal in natural systems, the activated sludge was fed 720 mg/l of phenol or 1,050 mg/l of methanol as TOC, using distilled water, tap water, ground water, and synthetic sea water as dilution water, without any other chemical aids.

Sampling and analytical procedures were the same as those described in the previous section. pH and temperature were measured periodically throughout the reaction periods.

Oxygen Requirement

In addition to all the procedures described above, oxygen consumption rates (as mg/l O_2/min) were measured. BOD bottles (300 ml) were filled with mixed liquor samples and dissolved oxygen changes with time were measured using an oxygen probe, which was designed to seal the BOD bottle during the measurement.

TEST RESULTS

Substrate removal and bacterial growth kinetics theory is well established and numerous papers in the literature describe how the kinetic coefficients can be evaluated using linear graphical methods. However, there appears to be a dearth of information on kinetic coefficients for specific substances. Moreover, use of the linear graphical methods leads one to wonder how reliable the reported evaluations are.

The shortcomings of the linear graphical methods are reviewed briefly and statistical methods are developed for evaluation of kinetic coefficients. Using the statistical methods, pH, salinity, temperature, and nutrient effects on substrate removal and bacterial growth kinetics are evaluated numerically and confidence intervals are given for the more important parameters evaluated. Oxygen requirements are evaluated based on the substrate remova! and bacterial growth kinetics study and checked against the experimental results, because the linear graphical method is apt to produce biased results. The reliability of substrate removal and bacterial growth kinetics data are discussed in detail by comparing the experimental results and the kinetic models.

Phenol

Kinetic Parameters —

The most widely used method for evaluating kinetic parameters (the substrate utilization rate coefficient (k), the Michaelis-Menten constant (K_S) the cell yield coefficient (a), and the cell decay coefficient (k_d)) is a linear graphical method in which Equations 14 and 15 are arranged in linear forms (Eckenfelder and Ford, 1970). Arrangement of Equation 14 with substitution of $\Delta S/\Delta t$ for dS/dt yields:

$$\frac{X}{\Delta S/\Delta t} = \left(-\frac{K_s}{k}\right)\left(\frac{1}{S}\right) - \frac{1}{k}. \tag{18}$$

Plotting $\frac{X}{\Delta S/\Delta t}$ vs. $\frac{1}{S}$, k and K_s are obtained from the slope and Y intercept of the best fit straight line. Similarly, Equation 15 yields:

$$\frac{\Delta X/\Delta t}{X} = -a\frac{\Delta S/\Delta t}{X} - k_{d}. \tag{19}$$

Then, a and k_d are evaluated in the same manner as k and K_s.

The shortcomings of the above method are as follows. First, there are inevitable experimental errors in the measurements of X (MLVSS) and S (TOC). Second, these errors are enlarged by estimating ΔX and ΔS. Third, the errors are further increased by replacing dX/dt and dS/dt with $\Delta X/\Delta t$ and $\Delta S/\Delta t$. Last, variable transformations $(X/(\Delta s/\Delta t)$, $1/S$, $(\Delta X/\Delta t)/X)$ result in further enlargement of error and cause non-symmetric error distributions whose variance changes with the magnitudes of the corresponding terms. As a result, without highly accurate measurements of X and S, it is hard to expect straight lines and the best fit straight lines do not necessarily mean the best interpretation of the experimental results.

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Under the experimental conditions described above, the expected errors of VSS measurements were almost constant in the VSS range of 200 to 4,000 mg/l and were 67 mg/l for phenol-acclimated sludge and 126 mg/l for methanol-acclimated sludge (estimated from Equation 28 below). Analysis of TOC was conducted in such a manner (mainly by repeating the sample injection into the analyzer) that the errors fell within 10 mg/l. With these errors, the plots of data according to the above equation were spread widely and there seemed to be little meaning in finding the best fit straight lines. An example that deals with the transformation of Equation 17 to the linear form $Rr/X = -a'((\Delta s/\Delta t)/X) + b'$, will be presented in Figure 34.

The following method is developed for the evaluation of kinetic parameters from batch test data. Rearrangement of Equation 15 yields:

$$dX = -a\,dS - k_d X\,dt\ . \tag{20}$$

Integration of Equation 20 from time t_o to t results in:

$$X - X_o = a(S_o - S) - k_d \int_{t_o}^{t} X dt. \tag{21}$$

If the time interval $(t - t_o)$ is not big enough to allow a dramatic change in X during the interval, $k_d \int_{t_o}^{t} X\,dt$ can be substituted by $k_d\bar{X}(t-t_o)$, where $\bar{X}$ denotes the mean biomass concentration during time t_o to t. Then, Equation 21 becomes:

$$X - X_o = a(S_o - S) - k_d\bar{X}(t-t_o) \tag{21'}$$

Under the previously described experimental conditions, the error caused by the left hand side of the equation $(X-X_o)$ is much greater than that by the right hand side of the equation. Thus, the terms in the right hand side of the equation may be regarded as exact variables. Assuming the observed values of $(X-X_o)$ are normally distributed around the true values of $(X-X_o)$ with a variance of σ^2, we obtain the likelihood function for Equation 21':

$$L = \frac{1}{(\sqrt{2\pi}\sigma)^N} \exp\left(\frac{\Sigma R_i^2}{2\sigma^2}\right) \tag{22}$$

where: L = likelihood,

σ = standard deviation of $(X-X_o)$,

$R_1 = (X-X_o) - a(S_o - S) - k_d\bar{X}(t-t_o)$, and (23)

N = number of observations.

The values of a and k_d, which provide the maximum likelihood values, are obtained from differentiation of Equation 22 with respect to k_d and a. From $\partial L/\partial a=0$ and $\partial L/\partial kd=0$, we get:

$$k_d = \frac{\Sigma(S_o-S)^2\,\Sigma\bar{X}(t-t_o)(X-X_o) - \Sigma(S_o-S)(X-X_o)\,\Sigma(S_o-S)(t-t_o)\bar{X}}{\{\Sigma\bar{X}(t-t_o)(S_o-S)\}^2 - \Sigma(S_o-S)^2\,\Sigma\{(t-t_o)\bar{X}\}^2} \tag{24}$$

and

$$a = \frac{\Sigma(X-X_0)(S_0-S) - k_d\Sigma\bar{X}(t-t_0)(S_0-S)}{\Sigma(S_0-S)^2} \qquad (25)$$

Since k_d and a are linear combinations of $(X-X_0)$, which is assumed to be normally distributed, they are also normally distributed. Their variances are given by:

$$\sigma^2_{k_d} = \sigma^2_{(x-x_0)} \frac{\{\Sigma(s_0-s)^2\}^2 \Sigma\{\bar{x}(t-t_0)\}^2 + \Sigma(s_0-s)^2\{\Sigma(s_0-s)\bar{x}(t-t_0)\}^2}{\left[\{\Sigma(s_0-s)\bar{x}(t-t_0)\}^2 - \Sigma(s_0-s)^2\,\Sigma\{\bar{x}(t-t_0)\}^2\right]^2} \qquad (26)$$

and

$$\sigma^2_a = \sigma^2_{(x-x_0)} \frac{\Sigma(s_0-s)^2}{\{\Sigma(s_0-s)^2\}^2} + \sigma^2_{k_d}\left\{\frac{\Sigma\bar{x}(t-t_0)(s_0-s)}{\Sigma(s_0-s)^2}\right\}^2 \qquad (27)$$

where:

$$\sigma^2_{(x-x_0)} = \frac{\Sigma\{(x-x_0)_o - (x-x_0)_e\}^2}{N-2} \qquad (28)$$

$(X-X_0)_o$ = observed value,
$(X-X_0)_e$ = estimated value from Equation 21', and
N = number of observations.

Combining Equations 14 and 20 and eliminating dt, we obtain:

$$dX = -a\,dS + k_d\left(\frac{K_s+S}{K_s}\right)dS. \qquad (29)$$

Integration of Equation 29 from time t_o to t yields:

$$X-X_0 = \left(a - \frac{k_d}{K}\right)(S_0-S) - \frac{k_dK_s}{K}\ln(S_0/S). \qquad (30)$$

If $(X-X_o)$ previously estimated from Equation 31 is used instead of $(X-X_o)$ observed, the variance is greatly reduced and the estimation of the coef-ficients, k and K_S, is significantly improved. The residual R_2 is expressed as:

$$R_2 = (X-X_o)_e - A\,(S_o - S) + B\ln(S_o/S), \tag{31}$$

where: $A = a - k_d/k$ and (32)

$$B = k_d K_S/k\ . \tag{33}$$

Solving for k and K_s using the least squares method:

$$B = \frac{\Sigma(S_o-S)^2\Sigma(X-X_o)_e\ln(S_o/S) - \Sigma(X-X_o)_e(S_o-S)\Sigma(S_o-S)\ln(S_o/S)}{\{\Sigma(S_o-S)\ln(S_o/S)^2 - \Sigma(S_o-S)^2\,\Sigma\ln(S_o/S)\}^2} \tag{34}$$

$$A = \frac{\Sigma(X-X_o)_e(S_o-S) + B\,\Sigma(S_o-S)\ln(S_o/S)}{\Sigma(S_o-S)^2} \tag{35}$$

Then, $k = k_d/(a-A)$ and (36)

$$K_s = Bk/k_d\ . \tag{37}$$

The variances of A and B are given as:

$$\sigma_B^2 = \sigma^2_{(x-x_o)_e}\frac{\{\Sigma(S_o-S)^2\}^2\,\Sigma\{\ln(S_o/S)\}^2 + \{\Sigma(S_o-S)\ln(S_o/S)\}^2\,\Sigma(S_o-S)^2}{\left[\{\Sigma(S_o-S)\ln(S_o/S)\}^2 - \Sigma(S_o-S)^2\,\Sigma\{\ln(S_o/S)\}^2\right]^2} \tag{38}$$

and

$$\sigma_A^2 = \sigma^2_{(x-x_o)_e}\frac{\Sigma(S_o-S)^2}{\{\Sigma(S_o-S)^2\}^2} + \sigma_B^2\frac{\{\Sigma(S_o-S)\ln(S_o/S)\}^2}{\{\Sigma(S_o-S)^2\}^2} \tag{39}$$

where

$$\sigma^2_{(x-x_o)_e} = \frac{\Sigma\{(x-x_o)'_e - (x-x_o)_e\}^2}{N-2} \tag{40}$$

$(X-X_o)_e$ = estimated value from Equation 30.

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The variance of K_s is given by:

$$\sigma^2_{K_o} = (k/k_d)^2 \sigma^2_B \tag{41}$$

and the variance of k_d/k is:

$$\sigma^2_{(k_d/k)} = \sigma^2_a + \sigma^2_A \tag{42}$$

The 80% confidence interval of k_d/k is:

$$\left\{\mu_{(k_d/k)} - 1.28\sigma_{(k_d/k)}\right\} \leq k_d/k \leq \left\{\mu_{(k_d/k)} + 1.28\sigma_{(k_d/k)}\right\} \tag{43}$$

Therefore, the 80% confidence interval of k is:

$$k \leq k_d/(\mu_{(k_d-k)} - 1.28\ \sigma_{(k_d/k)}) \text{ or} \tag{44}$$

$$k \geq k_d/(\mu_{(k_d+k)} + 1.28\ \sigma_{(k_d/k)}) , \tag{45}$$

if $\mu_{(k_d/k)} - 1.28\sigma_{(k_d/k)} < 0$, or

$$k_d/(\mu_{(k_d/k)} + 1.28\sigma_{(k_d/k)}) \leq k \leq k_d/(\mu_{(k_d/k)} - 1.28\sigma_{(k_d/k)}),$$

if $\mu(k_d/k) - 1.28\sigma_{(k_d/k)} > 0$. (46)

In order to reduce the error caused by the term $\ln(S_o/S)$ in Equation 30, the data from the reaction stage in which endogeneous respiration prevails over other activities are not used in this estimation. Estimated kinetic parameters using the above method are shown in Table 6. The standard deviation and confidence interval includes experimental errors and the variability of bacterial activities with biomass concentration, substrate concentration, and other effects. As shown in Table 6, K_s and a change with temperature insignificantly. Thus, it may be possible to conclude that the average values of K_s and a are the inherent characteristics of phenol waste that are not affected by temperature. Since hypothesis tests fail to disprove this assumption

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(see Appendix I, 1 and 2), all kinetic parameters can be re-evaluated based on the above theory.

TABLE 6. ESTIMATED KINETIC PARAMETERS FOR PHENOL

Temp (^oC)	Parameter	Expected value	Standard deviation	80% Confidence interval	X range (mg/1)	S range (mg/1)	N
5	K_s [1]	260.2	107.9				
	k [2]	0.02026		0.00630 0.01671	460 to 4,100	up to 650	65
	k_d [2]	0.002179	0.000838				
	a	1.258	0.077				
23	K_s [1]	240.9	171.2				
	k [2]	0.07177		0.01860 0.03863	470 to 4,100	up to 720	22
	k_d [2]	0.006755	0.001129				
	a	1.326	0.143				
28	K_s [1]	206.3	55.6				
	k [2]	0.06680		0.027 0.255	300 to 3,000	up to 790	29
	k_d [2]	0.005920	0.000999				
	a	1.048	0.069				

[1] Substrate concentrations are expressed as TOC (mg/1) throughout this study, if not specified otherwise.

[2] Expressed in units of hr^{-1}.

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The mean K_s and a values are:

K_s = 236 mg/l, σ_{K_s} = 70 mg/l and

a = 1.21, σ_a = 0.06

Corresponding k_d values are obtained from $\partial L/\partial kd = 0$, where L is the function defined in Equation 14; thus,

$$k_d = \frac{a\,\Sigma(S_o-S)\bar{X}(t-t_o) - \Sigma(X-X_o)\bar{X}(t-t_o)}{\Sigma\{\bar{X}(t-t_o)\}^2} \quad . \tag{47}$$

For a given value of cell yield coefficient, a, the variance of k_d is:

$$\sigma^2_{k_d} = \sigma^2_{(X-X_o)} \frac{1}{\Sigma\{\bar{X}(t-t_o)\}^2} \quad . \tag{48}$$

With a given K_s value, k is solved from $\partial\Sigma R_2^2/\partial k = 0$, where R_2 is the function given by Equation 21; thus,

$$k = k_d \frac{\Sigma(S_o-S)^2 + 2K_2\Sigma(S_o-S)\ln(S_o/S) + K_s^2\Sigma\{\ln(S_o/S)\}^2}{a\Sigma(S_o-S)^2 + aK_s\Sigma(S_o-S)\ln(S_o/S) - \Sigma(X-X_o)_e(S_o-S) - K_s\Sigma(X-X_o)_e\ln(S_o/S)} \tag{49}$$

For a given K_s, the variance of 1/k is given by:

$$\sigma^2_{(1/k)} = \frac{\sigma^2_{(X-X_o)_e}}{k_d^2} \frac{\Sigma(S_o-S)^2 + K_s^2\Sigma\{\ln(S_o/S)\}^2}{\left[\Sigma(S_o-S)^2 + 2K_s\Sigma(S_o-S)\ln(S_o/S) + K_s^2\Sigma\{\ln(S_o/S)\}^2\right]^2} \tag{50}$$

Thus, the 80% confidence interval of k is:

$$\frac{1}{\mu_{(1/k)} + 1.28\,\sigma_{(1/k)}} \leq k \leq \frac{1}{\mu_{(1/k)} - 1.28\,\sigma_{(1/k)}} \tag{51}$$

Re-estimated kinetic parameters corresponding to K_s = 236 mg/l and a = 1.21 are given in Table 7.

TABLE 7. KINETIC PARAMETERS FOR PHENOL CORRESPONDING TO K_s = 236 mg/l AND a = 1.21

Temperature (^oC)	Parameter (hr^{-1})	Expected value	Standard deviation	80% Confidence interval
5	k	0.01892		$0.01636 \le k \le 0.02306$
	k_d	0.002083	0.000591	
23	k	0.07068		$0.06188 \le k \le 0.08240$
	k_d	0.006487	0.000981	
28	k	0.07239		$0.96733 \le k \le 0.07828$
	k_d	0.006769	0.000962	

pH and Salinity Effects —

Using the previous method based on K_s = 236 mg/l and a = 1.21, the sub-strate removal rate coefficient (k) at various pH and salinity conditions was evaluated. The observed substrate removal rate coefficient at pH = 7 and salinity=0 ppt increased with increasing temperature from 0.9189 hr^{-1} at 5^oC to 0.0610 hr^{-1} at 21^oC and 0.0724 hr^{-1} at 28^oC. Let a substrate removal rate coefficient correction factor, f, denote the ratio of k at an arbitrary pH and salinity to k at pH = 7 and salinity = 0 ppt, as follows:

$$f = \frac{k}{k_{(pH = 7,\ sal = 0\ ppt)}} \tag{52}$$

Iso-f lines in pH and salinity coordinates are drawn through the experimental results and are presented in Figures 10, 11, and 12 for temperatures of 5, 21, and 28^oC, respectively.

In fresh water, the optimum pH range was 6.5 to 8.0. In this pH range, the minimum achievable f value was 0.9 at 5^oC and 0.8 at 21 and 28^oC. The bacterial activity decreased with salinity. In sea water (35 ppt salinity), the maximum achievable f value was 0.6 at 5^oC and 0.4 at 21 and 28^oC. As salinity increased, the optimum pH range changed. In sea water, the optimum pH range was 6 to 7 at 5^oC, 6.5 to 8 at 21^oC, and 7 to 8.5 at 28^oC.

Temperature Effects —

Based on the assumption that the substrate removal rate coefficient (k) changes with temperature according to Equation 16, the temperature coefficient, θ, is calculated to be 1.0760 at pH 7 and salinity 0 ppt in the temperature range of 5 to 23^oC. However, it is observed from Figures 10 through 12 that this value is not universal in all pH and salinity ranges. At low temperatures the pH and salinity

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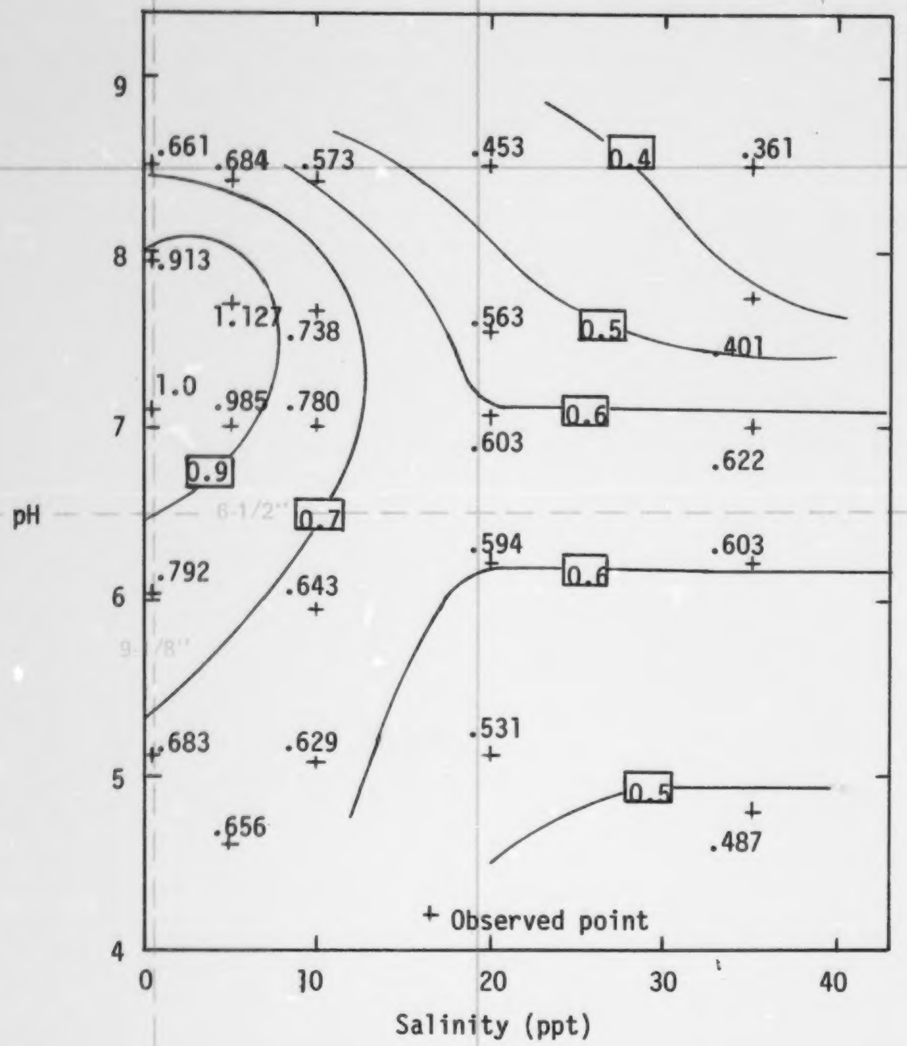

Figure 10. pH and salinity effects on the decomposition of phenol by acclimated sludge. Iso-f lines at 5 ^{o}C. *

* $k_{(pH=7,\ sal=0\ ppt,\ temp.=5\ ^{o}C)} = 0.0189\ hr^{-1}$

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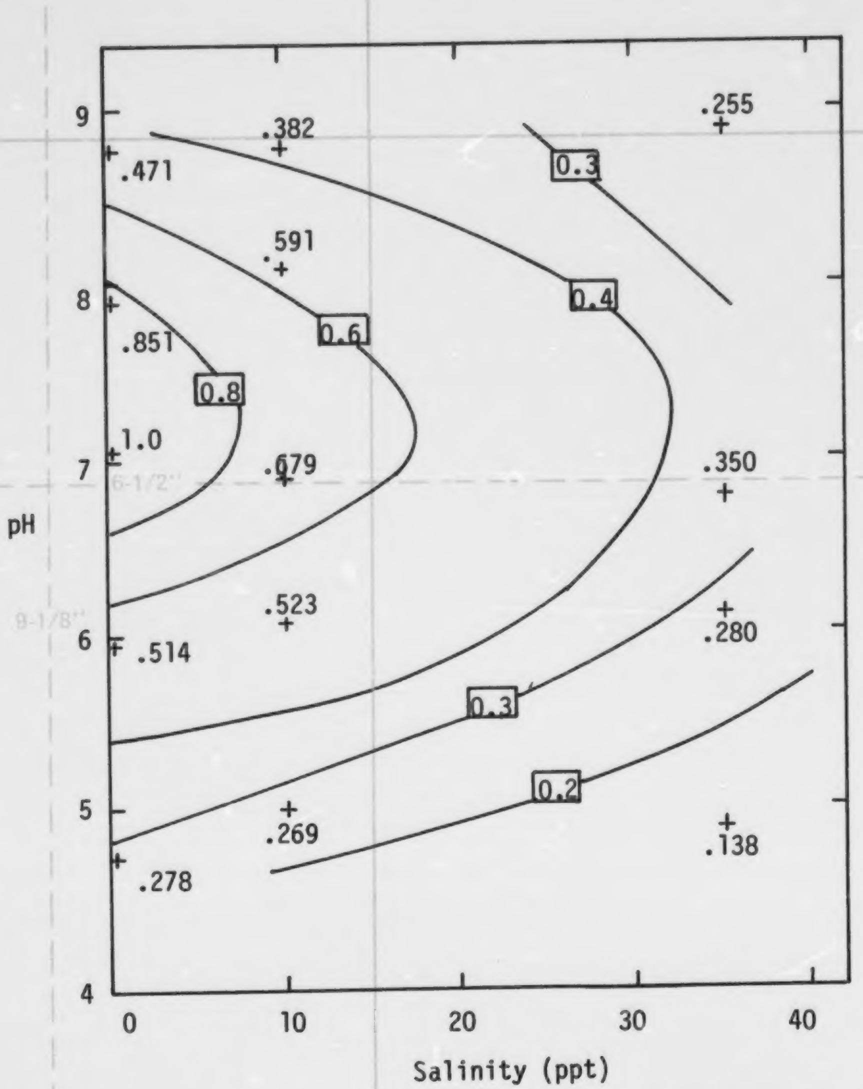

Figure 11. pH and salinity effects on the decomposition of phenol by acclimated sludge Iso-f linesat 21 ^{O}C.*

* k (pH=7, sal=0 ppt, temp.=21^{O}C)+0.0610 hr^{-1}

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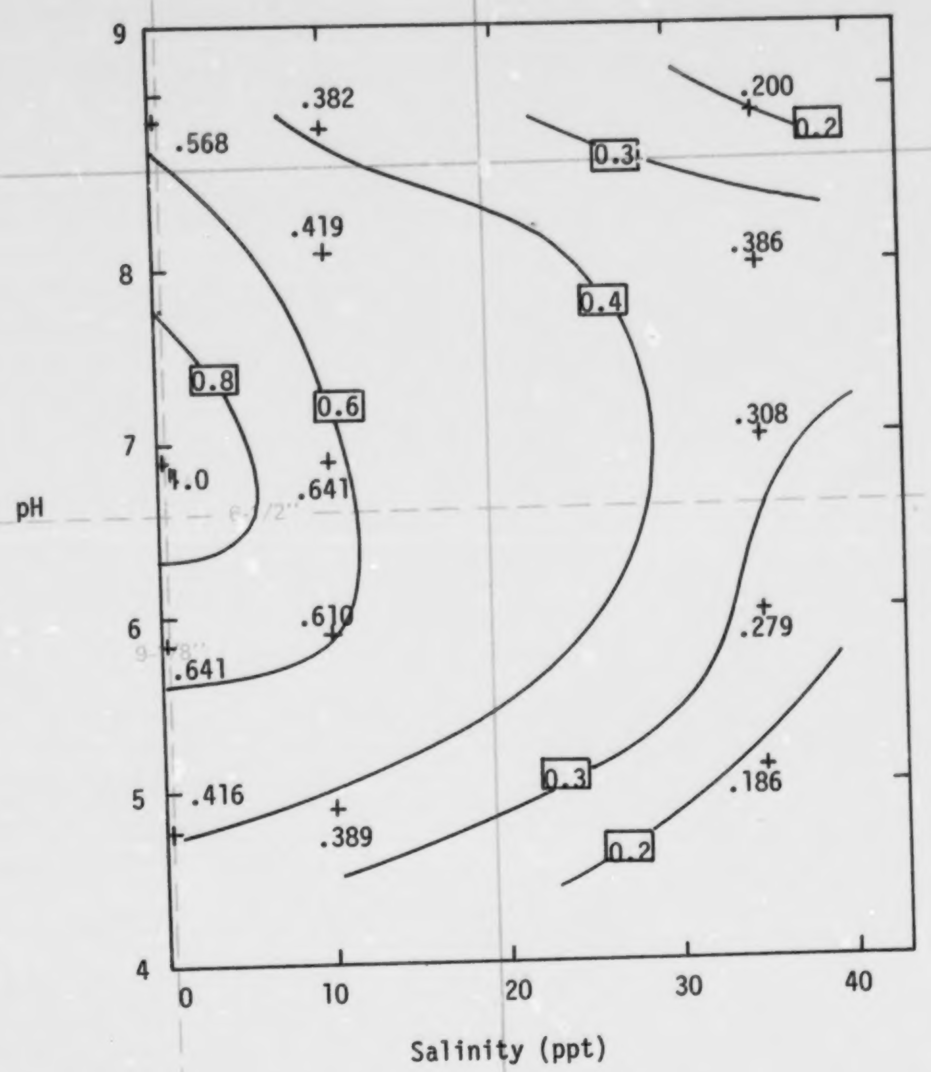

Figure 12. pH and salinity effects on the decomposition of phenol by acclimated sludge Iso-f lines at 28 ^{o}C.*

$^{*}k_{(pH=7,\ sal=0\ ppt,\ temp.=28\ ^{o}C)} = 0.0724\ hr^{-1}$

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effects are conspicuously decreased compared to those at high temperatures. This means that the temperature effect is decreased as pH and salinity become unfavorable to organisms. Iso-θ lines in the pH and salinity coordinates are presented in Figure 13. The calculated θ values ranged from 1.0145 at pH 5 and salinity 35 ppt to 1.0760 at pH 7 and salinity 0 ppt. In the pH and salinity range in which the temperature coefficient was greater than 1.06, the substrate removal rate coefficients at 23.5°C (computed from the modified Arrhenius equation) reached those values observed at 28°C, meaning that the temperature reached the optimum range for the organisms. In the range with θ less than 1.05, the θ values could be used to predict all k values in the test temperature range (5 to 28 °C). Thus, it may be concluded that as pH and salinity become more favorable to organisms, the temperature coefficient increases and the lower limit of the optimum temperature range is lowered.

Endogeneous Respiration —

The endogeneous respiration activity appeared to be affected by all the tested environmental factors, including temperature, pH, and salinity. With a limited accuracy in VSS analysis, a single batch reactor could not provide a reliable estimation of the cell decay coefficient, k_d. However, when the substrate removal rate coefficient, k, and the cell decay coefficient, k_d, were tabulated according to the ranges of k, k_d appeared to be correlated to k (see Table 8 and Figure 14). The following relationship between k and k_d may be assumed:

$$k_d = c_1 k^{c_2} \tag{53}$$

Taking the logarithm, the equation becomes:

$$\ln k_d = \ln c_1 + c_2 \ln k, \tag{53'}$$

where $\ln c_1$ and c_2 are the Y intercept and slope of the $\ln k_d$ vs. $\ln k$ graph (Figure 15), respectively. From Figure 15 the values $c_1 = 0.066$ and $c_2 = 0.87$ were obtained. The correlation coefficient between $\ln k_d$ and $\ln k$ was estimated to be 0.854. Thus, the following formula is proposed for the prediction of the cell decay rate coefficient of phenol-acclimated sludge:

$$k_d = 0.066\ k^{0.87}, \tag{54}$$

in which k and k_d have the units hr^{-1}.

Nutrient Effects on Substrate Removal --

Nitrogen and phosphorus addition did not display any detectable effect on either substrate removal patterns (Figures 16 and 17) or substrate removal rates (Table 9) in batch treatment using up to 700 mg/l of phenol as TOC, regardless of pH conditions and mineral supply. However addition of minerals (Mg^{++}, Mn^{++}, Fe^{++}, Ca^{++}, and Zn^{++}) showed a slightly perceptible effect on bacterial activity. At 20°C, when the pH was buffered, the average substrate removal rate coefficient was 0.0551 hr^{-1} with minerals compared to 0.0154 hr^{-1} without minerals.

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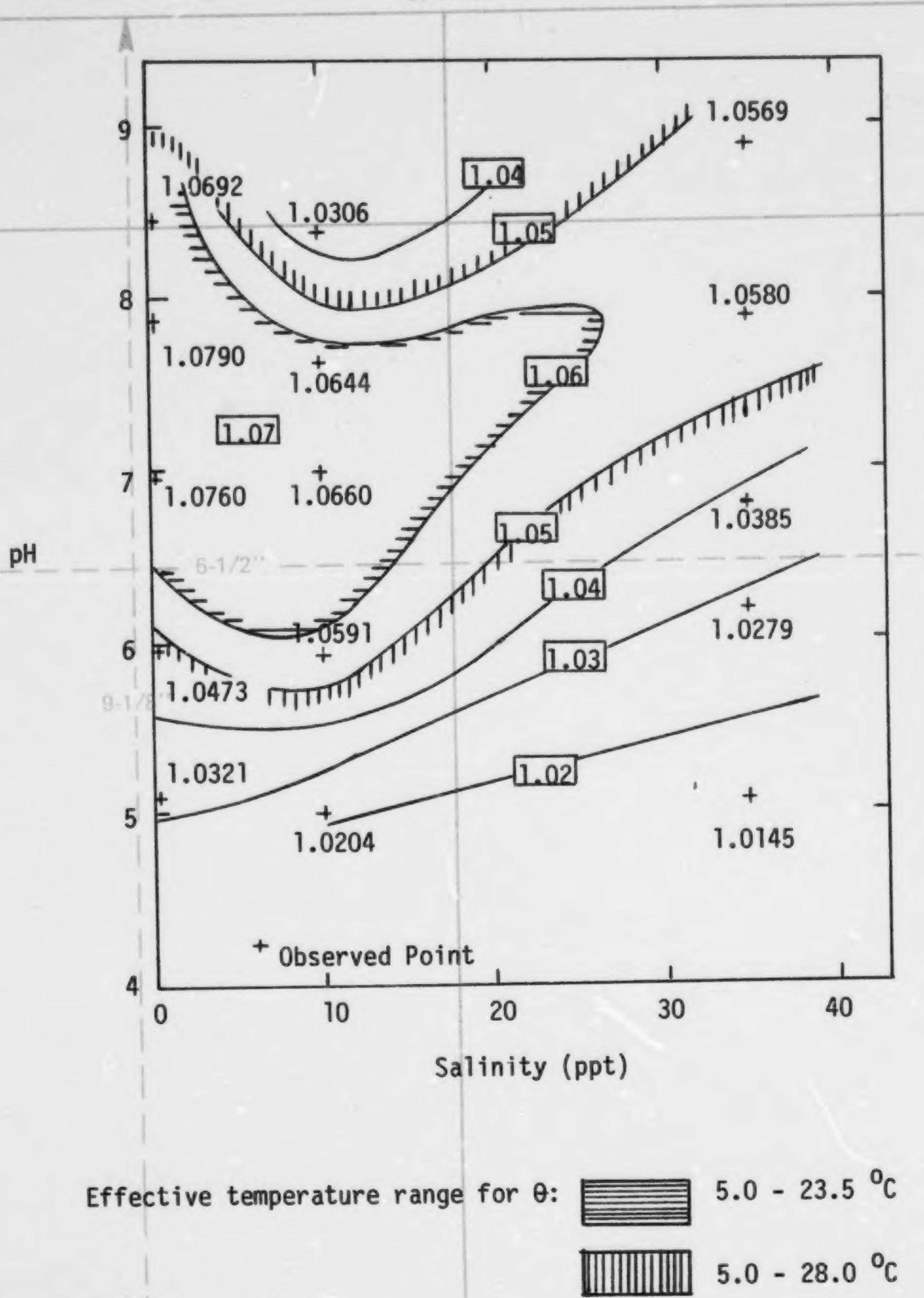

Figure 13. Temperature coefficient, θ, for the decomposition of phenol by acclimated sludge. Iso-θ lines.

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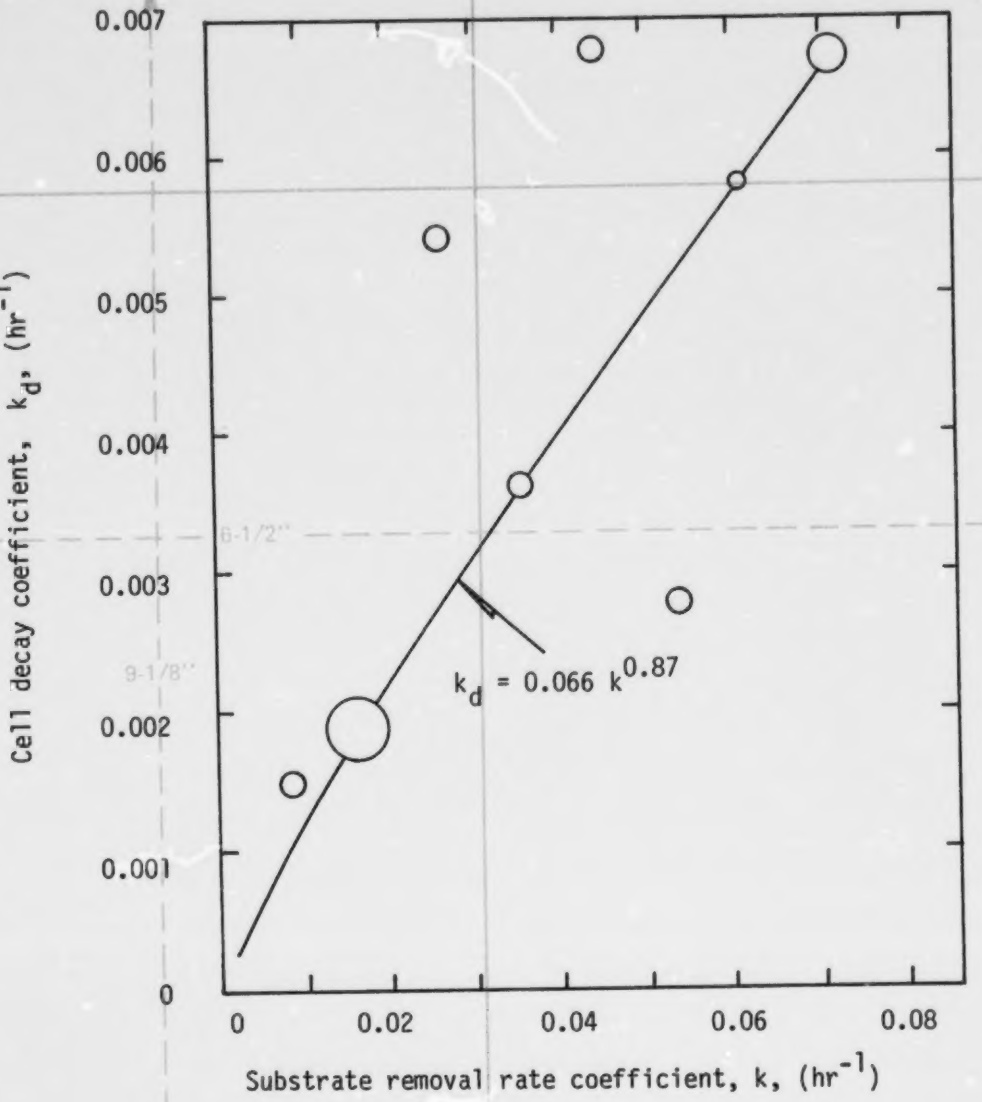

Figure 14. The relationship between the substrate removal rate coefficient and the cell decay coefficient for phenol acclimated sludge.

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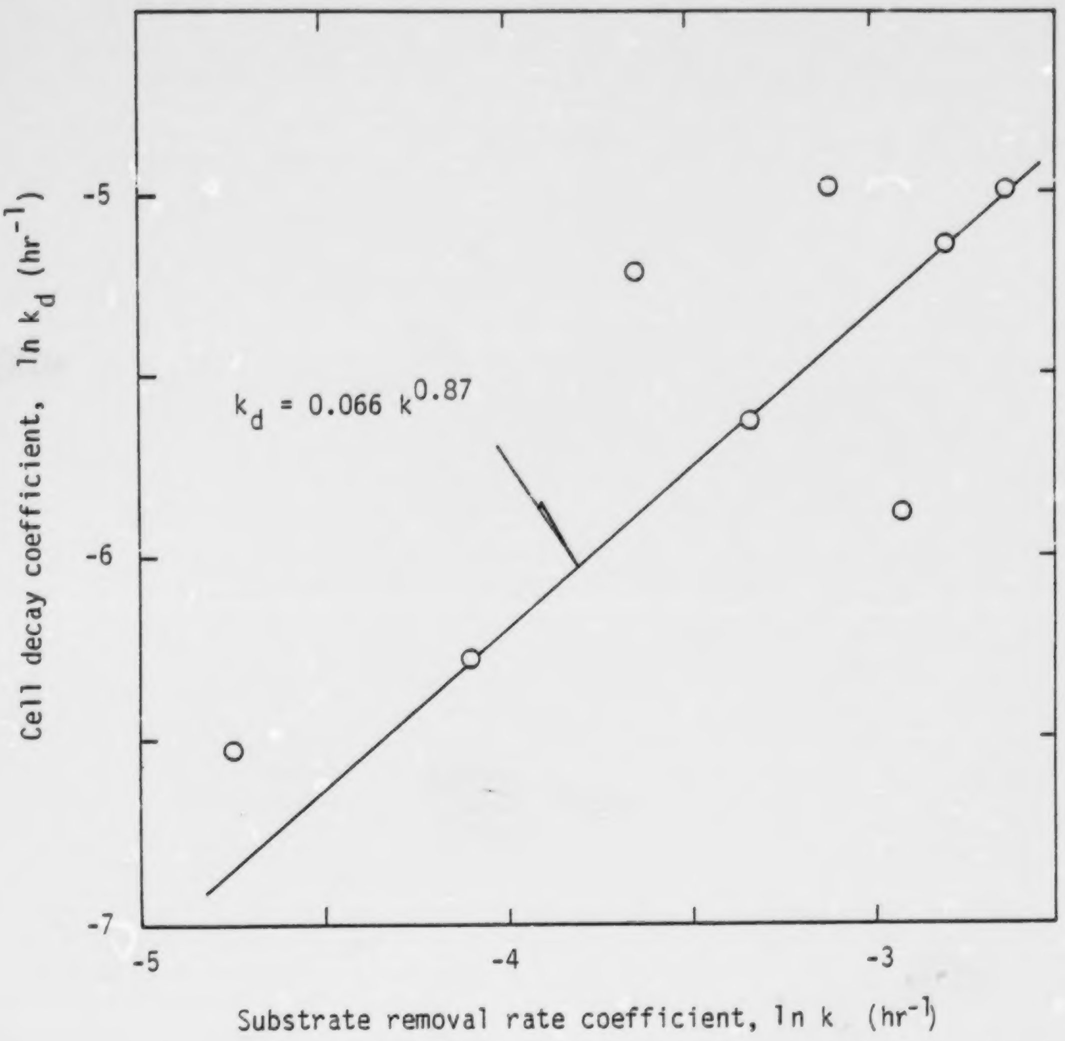

Figure 15. The relationship between the substrate removal rate coefficient and the cell decay coefficient for phenol acclimated sludge.

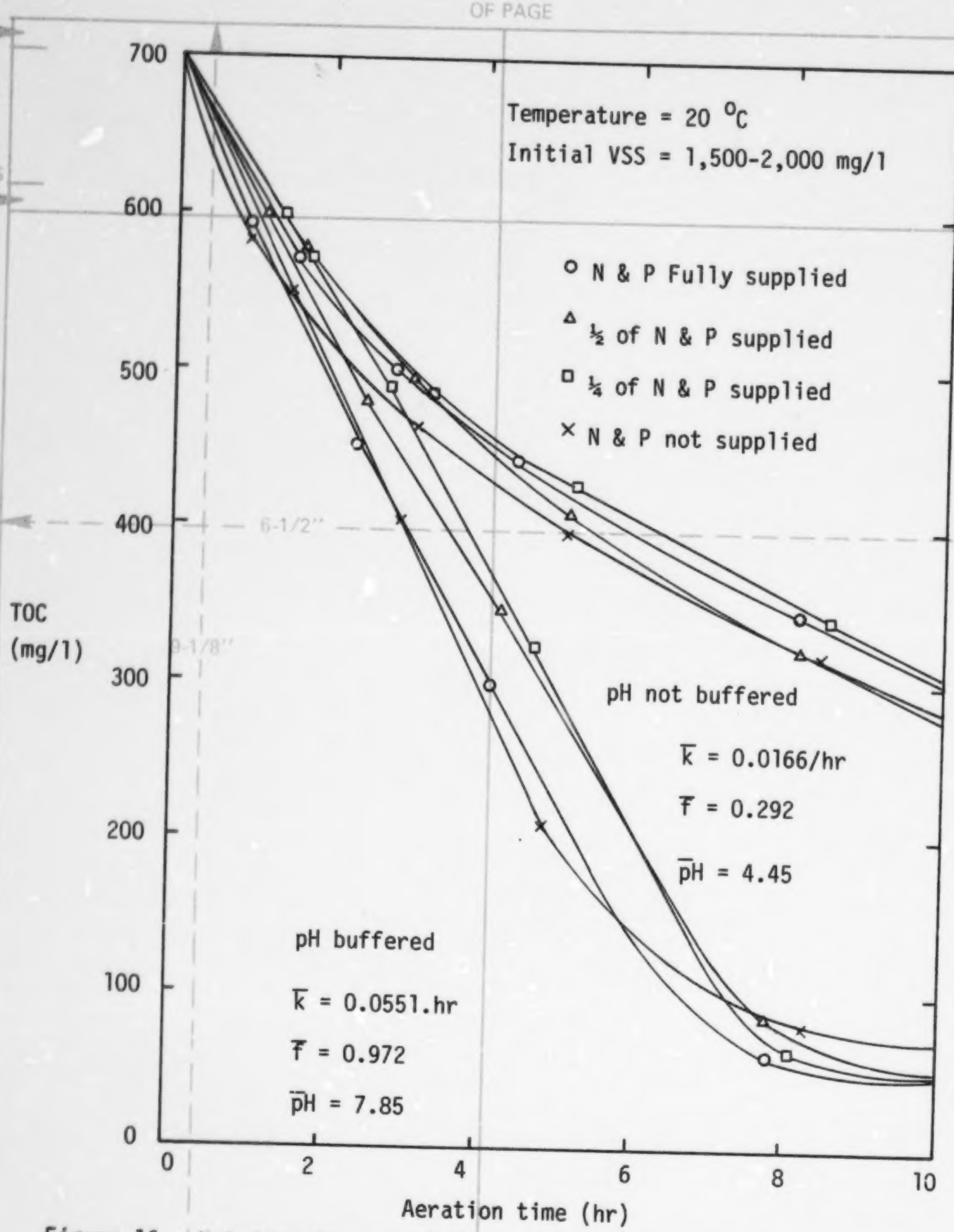

Figure 16. Nutrient (N & P) effects on the decomposition of phenol by acclimated sludge.
* Minerals supplied.

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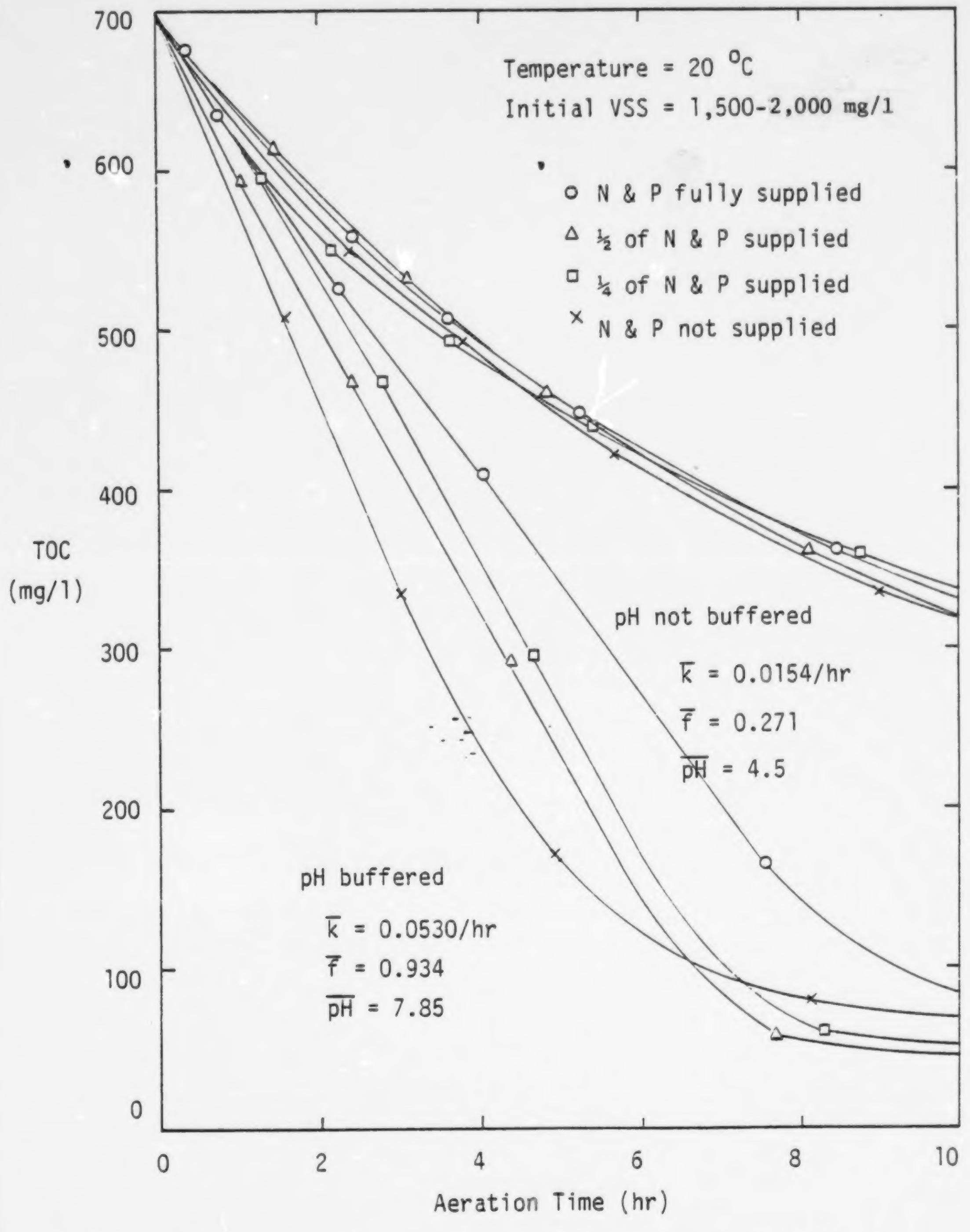

Figure 17. Nutrient (N & P) effects on the decomposition of phenol by acclimated sludge. Minerals not supplied.

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TABLE 8. THE RELATIONSHIP BETWEEN SUBSTRATE REMOVAL RATE COEFFICIENT AND CELL DECAY COEFFICIENT FOR PHENOL-ACCLIMATED SLUDGE

Range of k (hr^{-1})	Number of observations	Average k (hr^{-1})	Average k (hr^{-1})
0.00 - 0.01	5	0.00863	0.00145
0.01 - 0.02	41	0.01649	0.00186
0.02 - 0.03	8	0.02619	0.00541
0.03 - 0.04	6	0.03560	0.00358
0.04 - 0.05	5	0.04410	0.00676
0.05 - 0.06	9	0.05364	0.00276
0.06 - 0.07	2	0.06100	0.00582
0.07 - 0.08	15	0.07148	0.00671

Nitrogen and Phosphorus Effects on Cell Synthesis and Decay --

As shown in Equation 30, the net gain of cell mass is a function of the initial substrate concentration and the substrate concentration remaining. Therefore, if the values of kinetic coefficients (k, K_s, a, and k_d) are given, the net cell production can be predicted from the initial substrate concentration and the substrate concentration remaining, regardless of the initial biomass concentration.

In a normal growth pattern with nitrogen and phosphorus fully supplied, the cell decay coefficient, k_d, was found to be related to the substrate removal rate coefficient, k, as $k_d = 0.066\ k^{0.87}$ based on the data from 91 batch reactor tests. These estimated values of kinetic coefficients predicted the biomass growth with an expected error of 20 to 30 mg/l (based on Equation 40). When the biomass growth without nitrogen and phosphorus was compared to the normal growth, the following aspects, which are beyond the above error range, were observed (see Figures 18 and 19). First, there was more cell production at the initial growth phase (the biomass growth phase at substrate concentrations above 100 mg/l as TOC). This means that the cell yield coefficient is larger than 1.21 without nitrogen and phosphorus.

Wilkinson (1958) and Symons and McKinney (1958) reported the same result. Second, there was rapid cell decay at the end of the growth phase (substrate concentrations below 100 mg/l as TOC). It may be assumed that the cells that had been synthesized without extra-cellular nitrogen and phosphorus are more easily decayable than the normal cells that are synthesized with abundant nutrients. This cell decay rate appeared to increase when minerals were not supplied and when a more optimal pH condition was provided.

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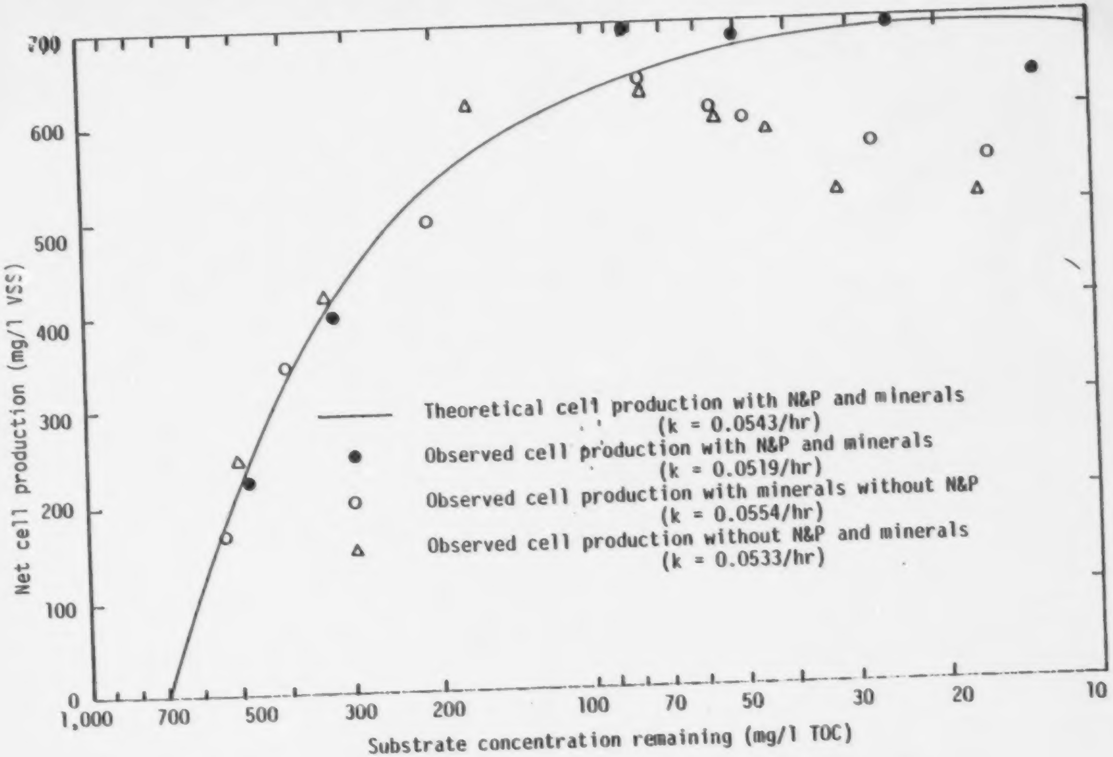

Figure 18. Cell synthesis without nitrogen and phosphorus. (I) pH buffered.

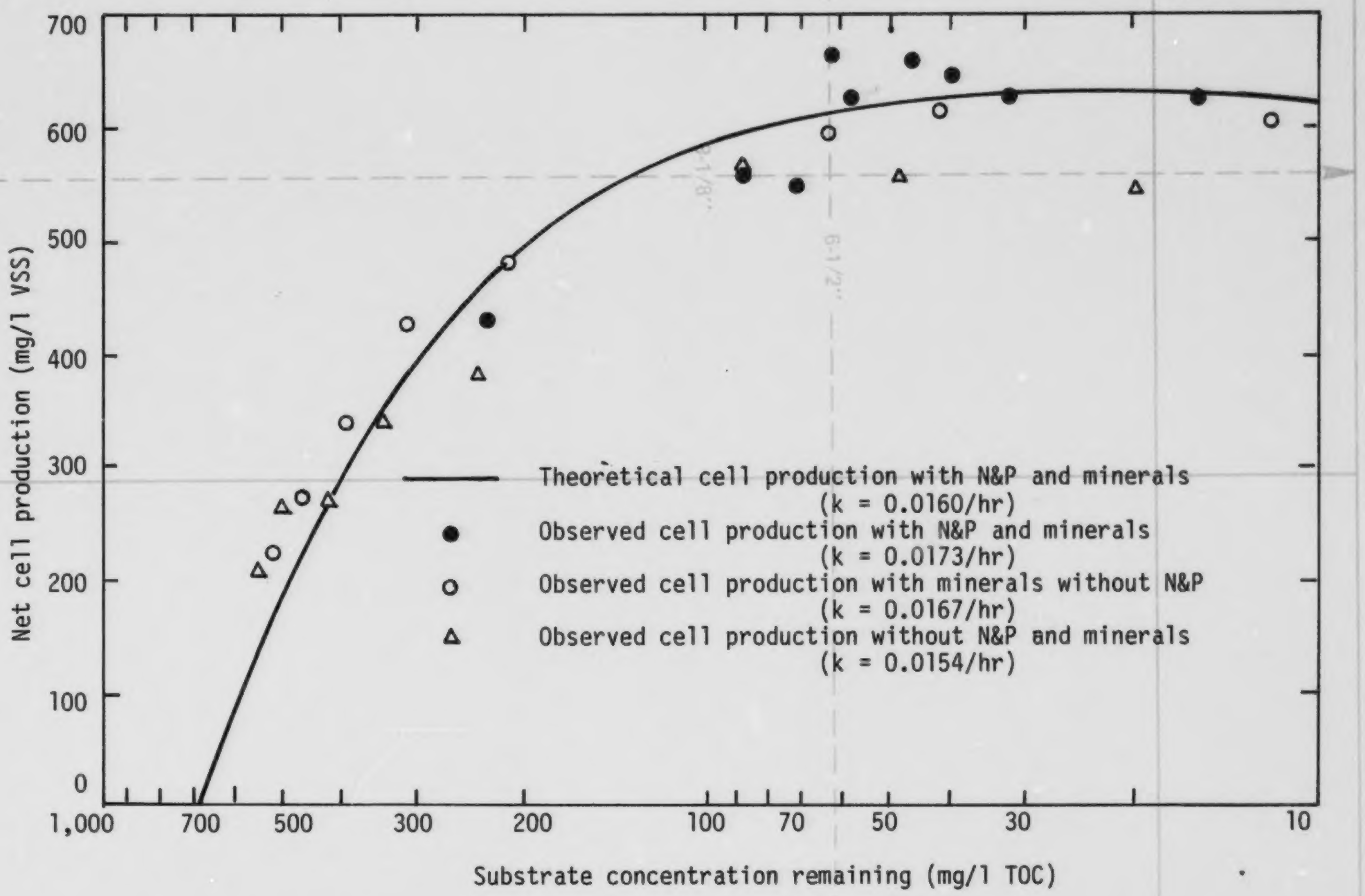

Figure 19. Cell synthesis without nitrogen and phosphorus. (II pH not buffered

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TABLE 9. NUTRIENT (N, P, AND MINERALS) EFFECTS ON THE DECOMPOSITION OF PHENOL BY ACCLIMATED SLUDGE[1]

pH Buffer (HCO_3^-)	Minerals	N&P	Avg. pH	pH Range	k (hr^{-1})	80% confidence interval of k	f
Yes	Yes	1	7.55	6.9-7.9	0.0568	0.0506-0.0647	1.002
		½	7.8	7.6-8.1	0.0547	0.0487-0.0622	0.965
		¼	7.95	7.6.8.4	0.0535	0.0469-0.0622	0.944
		0	8.1	7.6-8.6	0.0554	0.0491-0.0645	0.977
	No	1	7.6	7.2-7.9	0.0522	0.0475-0.0581	0.921
		½	7.8	7.6-8.1	0.0529	0.0469-0.0606	0.933
		¼	7.95	7.6-8.4	0.0535	0.0477-0.0609	0.944
		0	8.1	7.6-8.6	0.0533	0.0474-0.0610	0.940
		1	4.4	4.2-6.9	0.0166	0.0156-0.0177	0.293
		½	4.45	4.3-6.9	0.0165	0.0153-0.0179	0.291
No	Yes	¼	4.45	4.3-6.9	0.0164	0.0155-0.0175	0.289
		0	4.5	4.3-6.9	0.0167	0.0155-0.0181	0.295
	No	1	4.4	4.3-6.9	0.0152	0.0144-0.0161	0.268
		½	4.5	4.3-6.9	0.0153	0.0140-0.0168	0.270
		¼	4.45	4.4-6.9	0.0156	0.0148-0.0164	0.275
		0	4.6	4.5-7.3	0.0154	0.0124-0.0202	0.271

[1] Temp. = 20 °C, $K_{20^{\circ}C}$ = 0.0567 hr^{-1}.

TABLE 10. PHENOL DECOMPOSITION BY ACCLIMATED ACTIVATED SLUDGE IN NATURAL SYSTEMS WITHOUT CHEMICAL AIDS [1]

Test waters / Parameters	Distilled water	Tap water	Groundwater	Sea water
Initial pH	6.9	9.6	8.6	8.3
Alkalinity[2]		45	115	164
Hardness [2]		97	206	7,810
TDS (mg/l)		380	462	38,300
pH Range	4.5-7.3	4.6-9.6	5.4-8.6	4.95-8.3
Average k [3]	0.0154	0.0186	0.0592	0.0232
f	0.271	0.328	1.045	0.409
Equivalent pH	4.5	5.0	7.0	7.0

[1] Temperature = 20 °C; $k_{20^\circ C} = 0.0567\ hr^{-1}$

[2] Expressed as mg/l $CaCO_3$

[3] Average k (hr^{-1}) means the resultant k when approximately 90% of TOC removal was achieved.

Phenol Decomposition in Natural Systems without Chemical Aids --

Phenol decomposition resulted in a considerable decrease in pH. When the acclimated sludge was fed with 720 mg/l of phenol (as TOC) with no other chemicals, the pH dropped down from 6.9 to 4.5 in distilled water, from 9.6 to 4.6 in tap water with an alkalinity of 45 mg/l as $CaCO_3$, from 8.6 to 5.4 in groundwater with an alkalinity of 115 mg/l, and from 8.3 to 4.95 in sea water with an alkalinity of 164 mg/l. These unfavorable pH conditions lasted until nearly 90% TOC had disappeared, the removal results were equivalent to $k = 0.0154\ hr^{-1}$ in distilled water and $k = 0.0186\ hr^{-1}$ in tap water. These results are approximately the same as the results obtained at pH=4.5 and pH = 5, respectively, in a pH-regulated system. However, in groundwater and sea water, these temporary pH changes did not interfere with the phenol decomposition rates. The results were equivalent to those at pH 7 (see Figure 20 and Table 10).

Initial Lag Phase --

Even though the sludges were consistently fed with approximately 1,200 mg/l of TOC, less than 720 mg/l of TOC feeding caused initial lag phases in some cases. The lag phase was determined in the following way.

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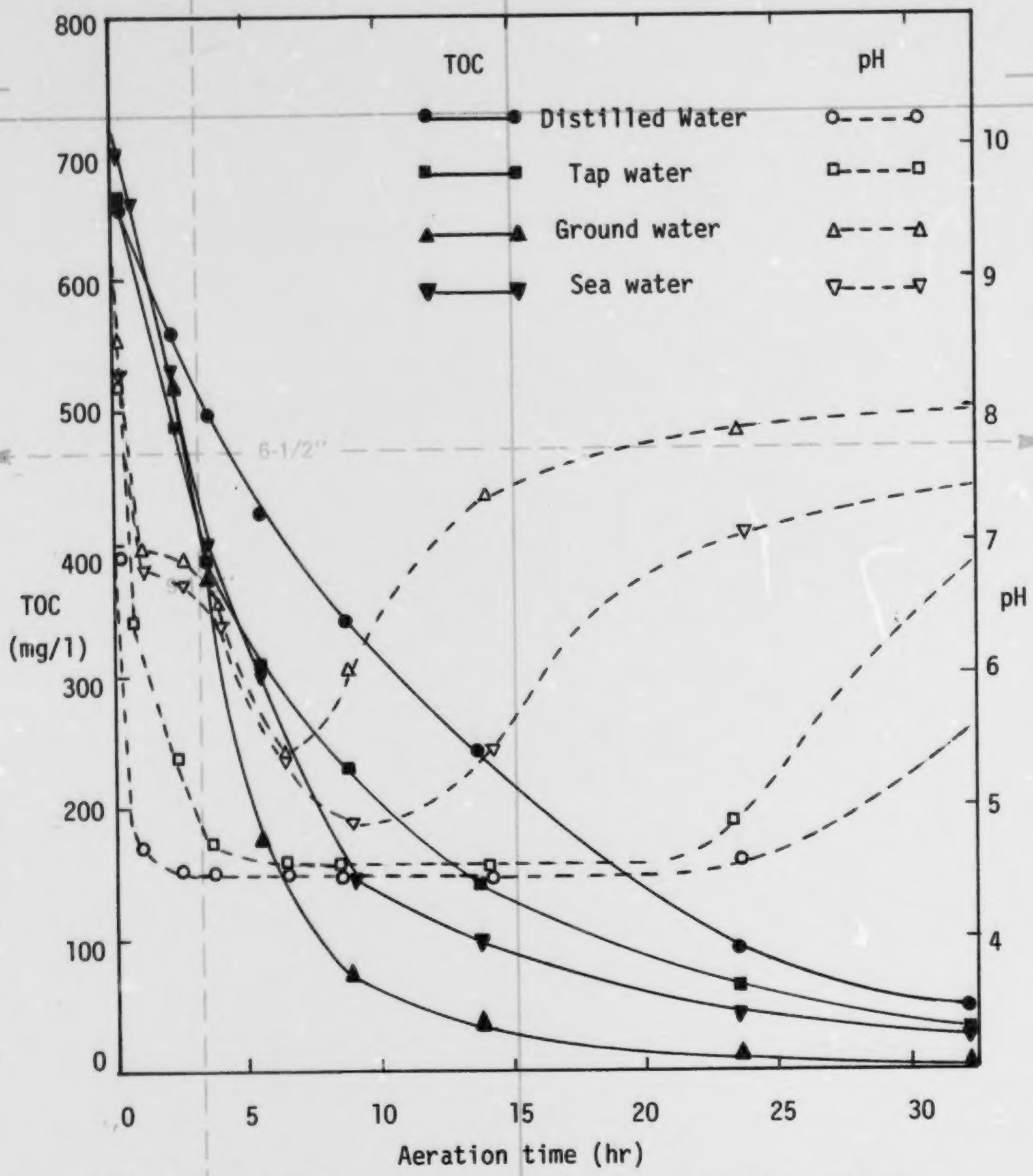

Figure 20. pH variations owing to the decomposition of phenol by acclimated activated sludge.

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If the biomass concentration, X, is considered as a constant, $\bar{X}$, between sampling times, then the integration of Equation 14 from time t_o to time t yields:

$$k = \frac{K_s \ln(S_o/S + (S_o-S)}{\bar{X}(t-t_o)} \qquad (55)$$

When the k values at initial stages were noticeably smaller than those at any other time intervals in a given reactor, these initial stages were considered to be in a lag phase. The above method was used to prevent experimental errors from affecting designation of the initial lag phase. Whether this lag phase in TOC removal really means a lag phase of bacterial activities, or is merely caused by decomposition of organics into other intermediates, will be discussed in the methanol study section.

Eight of 115 reactors displayed initial lag phases. In an extraordinary case, duration of the lag phase was about 32 hours, which caused a 15-hour aeration lag time. In the other seven cases, average duration of the lag phase was about four hours and the average aeration lag time was about three hours. To achieve a certain substrate concentration level at which organisms have recovered from the initial lag phase, a supplementary aeration time is required in addition to the theoretically calculated aeration time. This supplementary aeration time is designated to be the aeration lag time and is illustrated in Figure 21. Although the phenol-acclimated sludge, prior to the removal experiments, never experienced high salinities and high or low pH's, these new environments did not cause any particularly detectable lag phase. The experimental conditions and the initial lag phases are presented in Table 11.

TABLE 11. INITIAL LAG PHASE IN PHENOL DECOMPOSITION BY ACCLIMATED SLUDGE

Type of Test	Temp. (°C)	Number of tests	Cases of lag phase	Average duration (hrs)	Average lag time (hrs)	Remarks
Kinetic study	5	10	-	-	-	-
	23	8	5	5	4	pH=7
	28	6	-	-	-	sal=0 ppt
pH, salinity study	5	40	1	32	15	pH=5.1
	21	15	-	-	-	sal=0 ppt
	28	15	-	-	-	-
Nutrient study	20	20	1	2	1	Groundwater
Countermeasure application	28	1	1	2	2	Groundwater
Total	-	115	8	8	5	-

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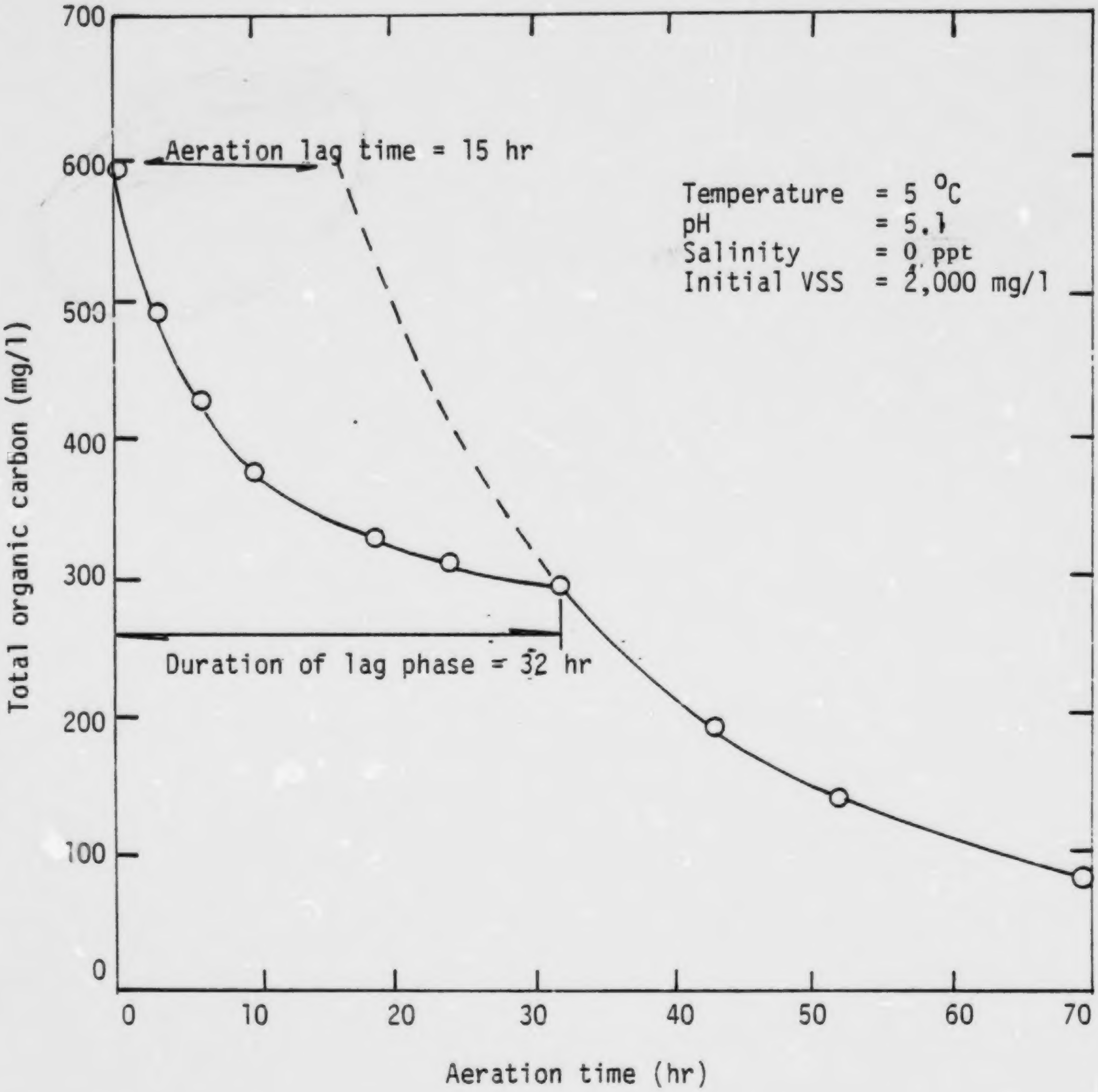

Figure 21. Initial lag phase in the decomposition of phenol by acclimated activated sludge.

Oxygen Requirement--

Previously, it was pointed out that removal of one gram of phenolic carbon resulted in production of 1.21 grams of organic solids (a = 1.21). Based on the approximate formulation of a bacterial cell (McKinney, 1962), $C_5H_7NO_2$, one gram of carbon produces 1.88 grams of organic cellular material, if it is used for synthesis. Therefore, 64.4% (1.21/1.88) of the phenol removed is calculated to be used for cell synthesis and the rest is completely oxidized to carbon dioxide and water.

Complete oxidation of phenol requires 3.11 grams of oxygen per gram of carbon according to:

$$\underset{(72)}{C_6H_5OH} + \underset{(224)}{7\,O_2} = 6\,CO_2 + 3\,H_2O. \qquad (56)$$

Complete oxidation of the cell requires 2.67 grams of oxygen per gram of cell carbon (or 1.42 grams of oxygen per gram of organic fraction of cell) according to:

$$\underset{(60)}{C_5H_7NO_2} + 5\,O_2 = \underset{(160)}{5\,CO_2} + NH_3 + 2\,H_2O\,. \qquad (57)$$

Therefore, one gram of carbon will require 0.44 grams (3.11-2.67 grams) of oxygen when it is utilized for cell synthesis. Thus, per gram of carbon, 64.4% of TOC removed requires 0.44 grams of oxygen and 35.6% of TOC removed requires 3.11 grams of oxygen.

The following estimation of the coefficients, a' and b', in Equation 17 are possible:

$$a' = (0.644 \times 0.44) + (0.356 \times 3.11) = 1.39 \text{ and } b' = 1.42 \times k_d.$$

Then, the oxygen utilization rate is expressed as:

$$Rr = -1.39\,\frac{dS}{dt} + 1.42\,k_d\,X, \qquad (58)$$

where dS/dt and k_d are given in Equations 14 and 54, respectively.

The oxygen uptake rates observed are compared with those computed from Equation 58 in Table 12. Through a hypothesis test, it may be proved that a' = 1.39 and b' = 1.42 k_d provide a reliable estimation of oxygen demand (see Appendix 1, 3).

Modification and arrangement of Equation 17 yields:

$$\frac{Rr}{X} = -a'\,\frac{\Delta S/\Delta t}{X} + b'\,. \qquad (58')$$

This is a linear equation and when the Rr/X vs. - (ΔS/Δt)/X graph is plotted, the slope is a' and the Y intercept is b'. The plots from experimental results are compared with Equation 58 in Figure 22. The line defined by Equation 58 is divided by the observed points equally and seems quite reasonable. More intensive evaluation was done in the methanol study.

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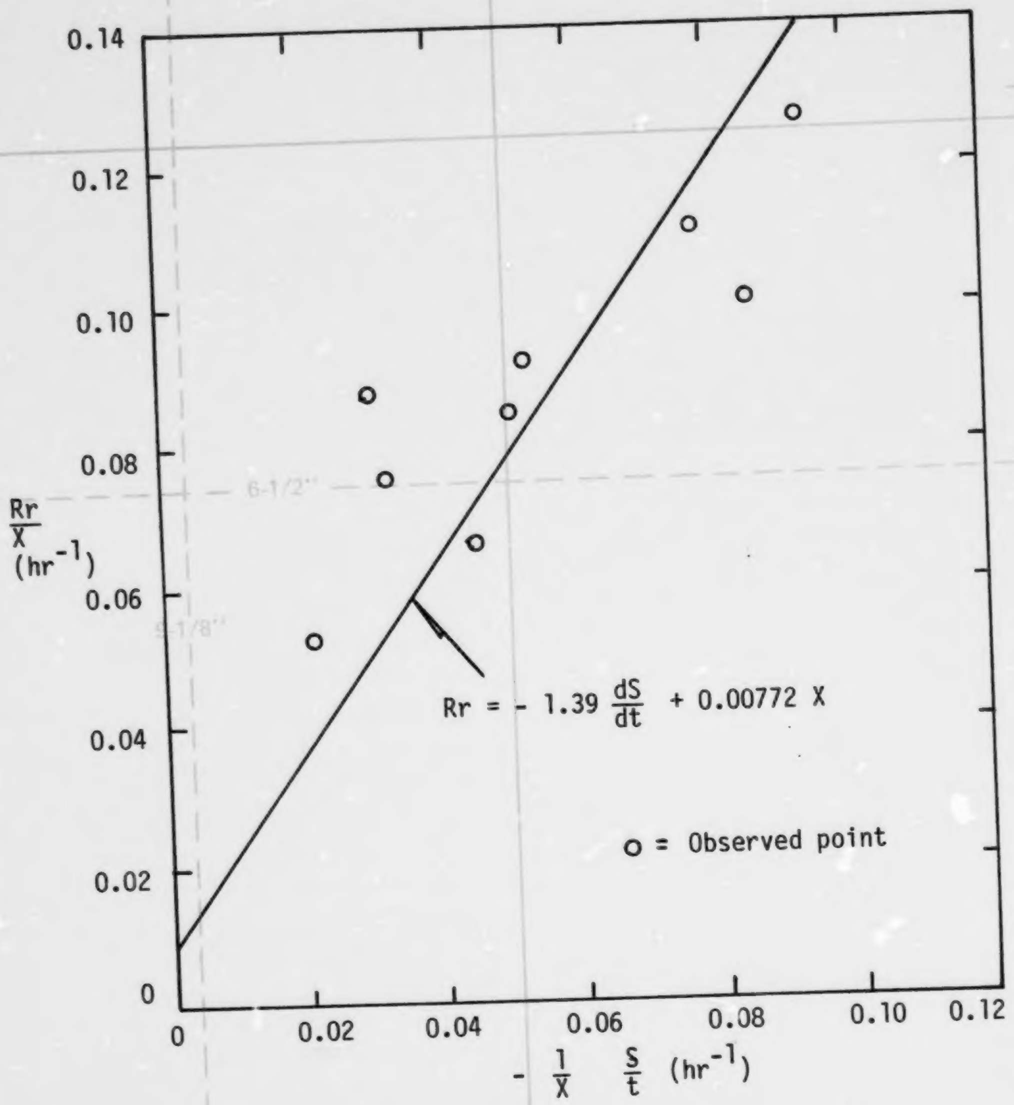

Figure 22. Comparison of theoretical and observed oxygen uptake rates in the decomposition of phenol by acclimated activated sludge.

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TABLE 12. COMPARISON OF THEORETICAL AND OBSERVED OXYGEN UPTAKE RATES IN PHENOL DECOMPOSITION BY ACCLIMATED SLUDGE [1]

O_2 Uptake rate observed Rr_1(mg/l/hr)	O_2 Uptake rate estimated Rr_2(mg/l/hr)	$Rr_1 - Rr_2$ [2]
22.5	15.9	6.6
27.6	16.0	11.6
34.1	43.4	-9.3
46.1	50.4	-4.3
8.7	6.4	2.3
16.7	15.0	1.7
17.3	16.0	1.3
13.8	14.6	-0.8
22.8	23.5	-0.7
0	1.1	-1.1

[1] Number of observations = 10.

[2] Mean =0.73; Standard deviation = 5.68.

Methanol

Kinetic Parameters —

Aeration of methanol resulted in a considerable amount of methanol loss by stripping. The rate of methanol stripping may be assumed to be a first order reaction, expressed as:

$$\frac{ds}{dt} = - k_e S \tag{59}$$

where k_e is the stripping rate coefficient (time^{-1}). (Further discussion of methanol stripping is contained in Appendix II). When methanol removal is accomplished by both stripping and biological decomposition, the removal rate is expressed as:

$$\frac{dS}{dt} = - \frac{kXS}{K_s + S} - k_e S \tag{60}$$

and the bacterial growth rate is:

$$\frac{dX}{dt} = -a\left(\frac{dS}{dt} + k_e S\right) - k_d X \ . \tag{61}$$

Integration of Equation 61 from time t_o to time t yields:

$$x - x_o = a(S_o - S) - a\ k_e \int_{t_o}^{t} S\ dt - k_d \int_{t_o}^{t} X\ dt. \tag{62}$$

When the time interval $(t-t_o)$ is too short for large changes to occur in S and X, $\int_{t_o}^{t} S\,dt$ and $\int_{t_o}^{t} X\,dt$ can be replaced by $\bar{S}(t-t_o)$ and $\bar{X}(t-t_o)$, respectively, where $\bar{S}$ and $\bar{X}$ denote the mean concentrations of S and X between times t_o and t, and the following equation results:

$$X-X_o = a\left\{(S_o-S) - k_e\bar{S}(t-t_o)\right\} - k_d\bar{X}(t-t_o). \tag{62'}$$

Equation 62' is the same as Equation 21' except that $(S_o - S)$ is replaced by $\left\{(S_o - S) - k_eS\ (t-t_o)\right\}$. Equations 24 through 27, with this substitution, provide the solution for the expected values and variances of a and k_d.

Let $dU/dt = dS/dt + k_eS$, (63)
then, Equation 60 becomes $dU/dt = -(kXS)/K_s+S)$. (64)

Thus, $dt = dU\,(K_s+S)/kXS$, (64')

and Equation 61 becomes $dX/dt = -a\,dU/dt - k_dX$. (65)

Combining Equations 63, 64, and 65, we get:

$$\frac{dX}{dt} = -a\,dU + \frac{k_d(K_s+S)}{kS}\,dU \tag{66}$$

$$= -(a-\frac{k_d}{k})\ dU + \frac{k_dK_s}{k}\left(\frac{dS}{S} - k_e\,dt\right) \tag{67}$$

Integration of Eq. 67 from time t_o to t yields:

$$X-X_o = (a-\frac{k_d}{k})(U_o-U) - \frac{k_dK_s}{k}\left\{\ln(S_o/S) - k_e\,(t-t_o)\right\} \tag{68}$$

In a short time interval, U_o-U can be expressed as:

$$U_o - U = (S_o - S) - k_e\bar{S}\,(t-t_o), \tag{69}$$

where $(U_o - U)$ is the amount of substrate removed by biological decomposition. Then, Equation 68 becomes:

$$X-X_o = (a-\frac{k_d}{k})\left\{(S_o - S) - k_e\,\bar{S}\,(t-t_o)\right\} - \frac{k_dK_s}{k}\ln(S_o/S) - k_e(t-t_o)\} \tag{69'}$$

Equations 34 through 39, with substitutions of $(S_o - S)$ and $\ln(S_o/S)$ by $\left\{(S_o - S) - k_e\bar{S}\,(t-t_o)\right\}$ and $\left\{\ln(S_o/S) - k_e(t-t_o)\right\}$ provide the solution for the expected values and variances of k and K_s.

When stripping is the only cause of methanol removal, the concentration at a given time is obtained by integrating Equation 59, which gives

$$S = S_o\exp(-k_et). \tag{70}$$

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Plotting ln S as the ordinate and t as the abscissa, k_e is obtained from the slope of the straight line.

When 1 liter of air per minute was supplied per liter of water, the stripping rate coefficients were estimated to be 0.00330, 0.00948, and 0.0277 hr^{-1} at 5, 22, and 28 °C, respectively. At these stripping conditions, the estimated kinetic parameters are shown in Table 13.

TABLE 13. ESTIMATED KINETIC PARAMETERS FOR METHANOL

Temp. (°C)	Parameter	Expected value	Standard deviation	80% Confidence interval	X Range (mg/1)	S Range (mg/1)	N
5	K_s[1]	2,587	2,747				
	k[2]	0.03754		≥0.00.09 ≤0.00116	200 to 1,300	up to 1,000	21
	k_d[2]	-0.001981	0.007850				
	a	1.013	1.313				
22	K_s[1]	-696	1,025				
	k[2]	-0.05376		≥0.00815 ≤0.01169	450 to 1,400	up to 920	8
	k_d[2]	0.0038338	0.003074				
	a	1.279	0.158				
28	K_s[1]	2,080	674				
	k[2]	0.1902		≥0.0162 ≤0.0195	200 to 1,600	up to 1,150	40
	k_d[2]	0.006758	0.005910				
	a	1.444	0.291				

[1] Expressed in units of mg/l.

[2] Expressed in units of hr^{-1}.

It is likely that the distribution of the estimated parameters was spread widely because of inconsistent methanol stripping conditions from reactor to reactor. At 22 °C, k and K_s were estimated to be negative; however, negative values for k and K_s are contradictory in Equation 13 and it is believed that not enough observations and some unfavorable experimental errors are the causes. Excluding these erroneous estimations, the kinetic coefficients, K_s and a, changed with temperature insignificantly. Thus, all kinetic parameters can be re-evaluated based on the assumption that the average values of K_s and a are the inherent characteristics of methanol that are not affected by temperature (cf. Appendix I, 1 and 2). The mean K_s and a that characterize methanol wastes are: K_s = 2,330 mg/l, σ_{k_s} = 1,410 mg/l and a = 1.25, σ_a=0.45. Re-estimated kinetic parameters corresponding to K_s = 2,330 mg/l and a = 1.25, using equations 47 through 51 with the previously described substitutions, are given in Table 14.

TABLE 14. KINETIC PARAMETERS FOR METHANOL CORRESPONDING TO K_s = 2,330 mg/l AND a = 1.25

Temperature (°C)	Parameter (hr^{-1})	Expected value	Standard deviation	80% Confidence interval
5	k	0.04407		$0.04034 \le k \le 0.04854$
	k_d	-0.000901	0.001524	
22	k	0.2805		$0.2213 \le k \le 0.3830$
	k_d	0.003617	0.002221	
28	k	0.2656		$0.2340 \le k \le 0.3057$
	k_d	0.005373	0.004642	

pH and Salinity Effects --

Methanol-acclimated activated sludge was insensitive to high pH in fresh water (see Figures 23, 24, and 25). At 5 °C, methanol removal rates were higher at pH 8 and 9 than at pH 7. At higher temperatures, the removal rate was still higher at pH 9 than at a pH less than 6.0. In sea water (35 ppt salinity), the maximum achievable f value was 0.7 at 5 °C, 0.1 at 22 °C, and 0.85 at 28 °C. Increasing salinity narrowed the tolerable pH range and reduced the f values correspondingly.

Temperature Effects --

Methanol-acclimated sludge was sensitive to temperature. Between 5 and 22 °C, the substrate removal rate tripled for a 10 °C increase (θ = 1.115) at pH 7 in fresh water, while there was no significant increase (θ = 1.0) in sea water. Between 22 and 28 °C, the temperature coefficient, θ, was 1.0 at pH 7 in fresh water, while it was 1.4 in sea water (see Figures 26 and 27). Thus, it may be deduced that first, the preferred temperature range for methanol sludge is very limited. Second, bacterial activity rapidly decreases at temperatures below the optimum temperature range. Third, bacterial activity is not greatly affected by temperature change within the optimum temperature range. Fourth, the lower limit of the optimum temperature range is lowered as other environmental factors (pH and salinity) become more favorable for the organisms. Similar results were observed for the phenol studies, but to a lesser extent.

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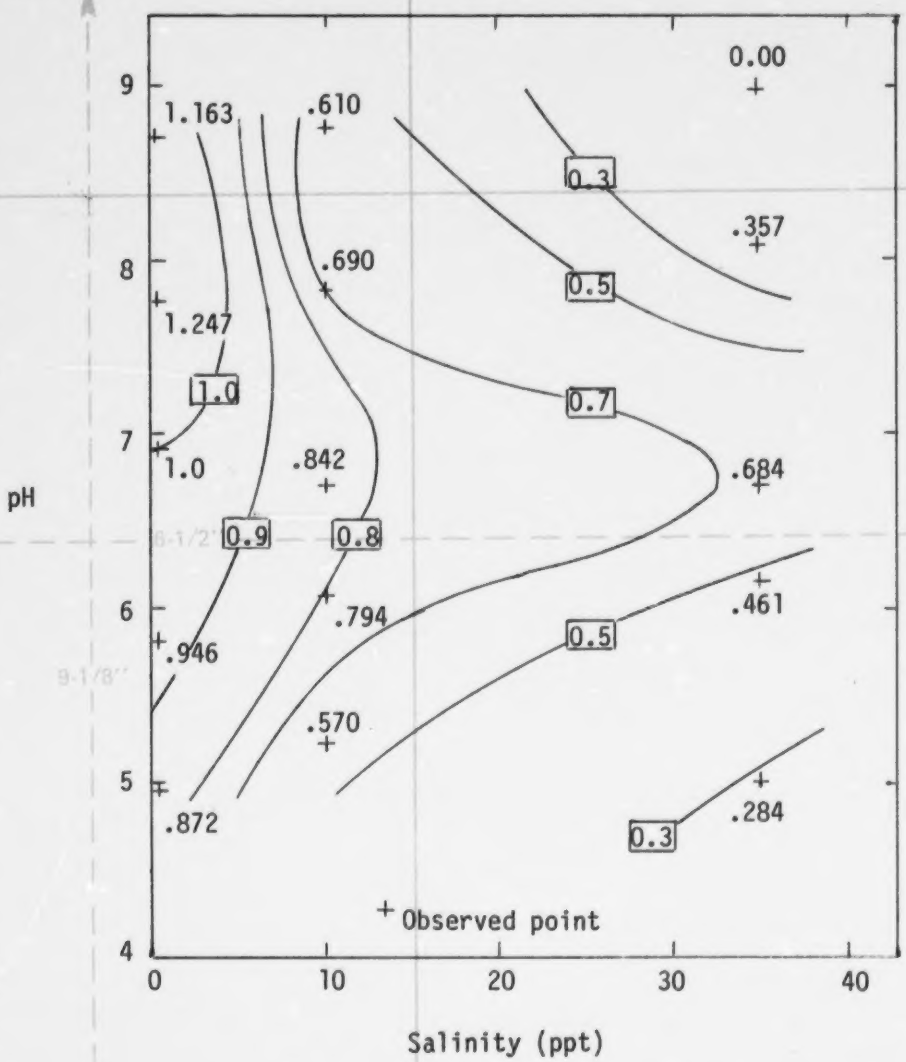

Figure 23. pH and salinity effects on the decomposition of methanol by acclimated sludge. Iso-f lines at 5 °C.

$k_{(pH=7,\ sal=0\ ppt,\ temp.=5\ ^{o}C)} = 0.04407\ hr^{-1}$

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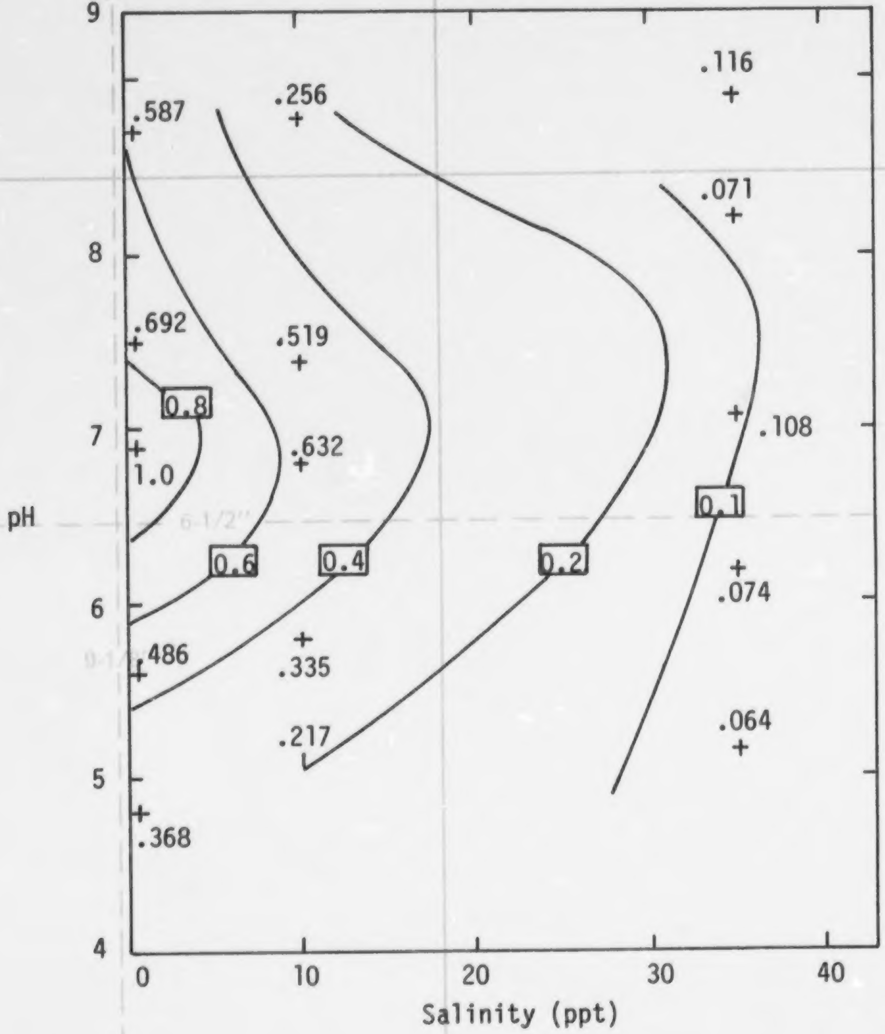

Figure 24. pH and salinity effects on the decomposition of methanol by acclimated sludge Iso-f lines at 22 °C.

$k_{(\mathrm{pH}=7,\ \mathrm{sal}=0\ \mathrm{ppt},\ \mathrm{temp.}=22\ ^{\circ}\mathrm{C})} = 0.2805\ \mathrm{hr}^{-1}$

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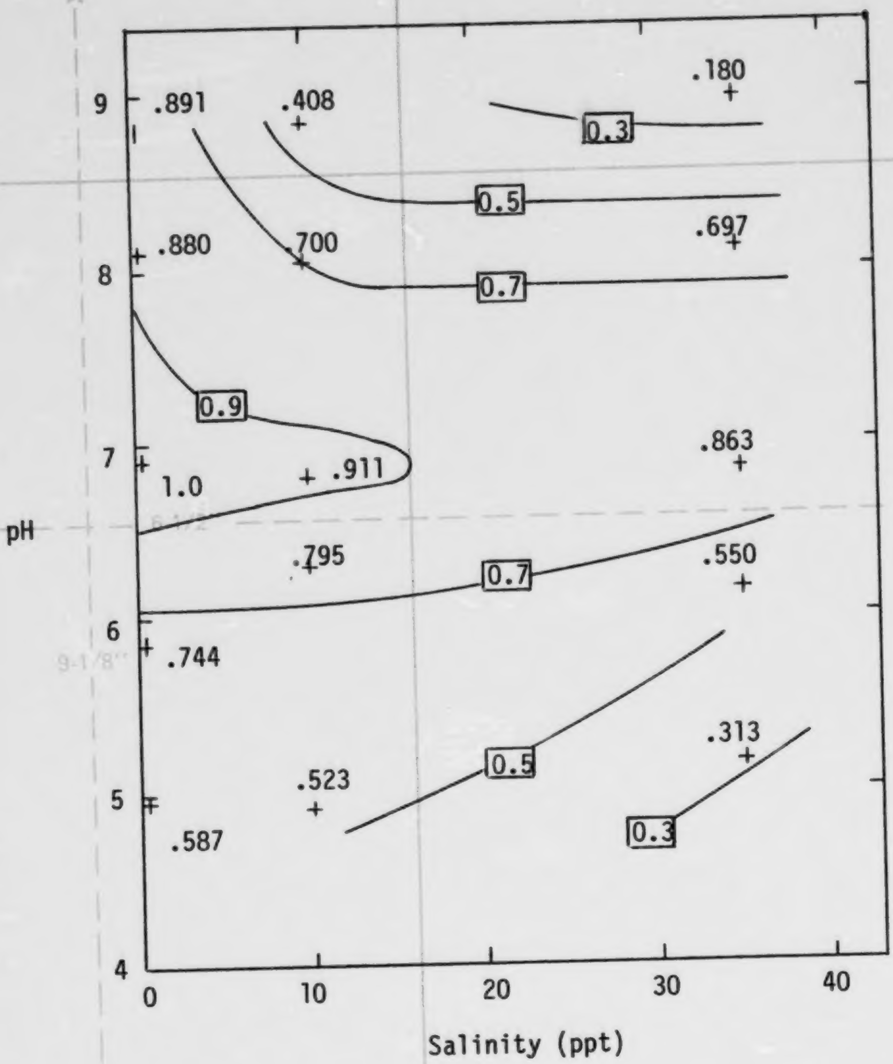

Figure 25. pH and salinity effects on the decomposition of methanol by acclimated sludge. Iso -f lines at 28 °C.

$$k_{(pH=7,\ sal=0\ ppt,\ temp.=28\ ^{o}C)} = 0.2656\ hr^{-1}$$

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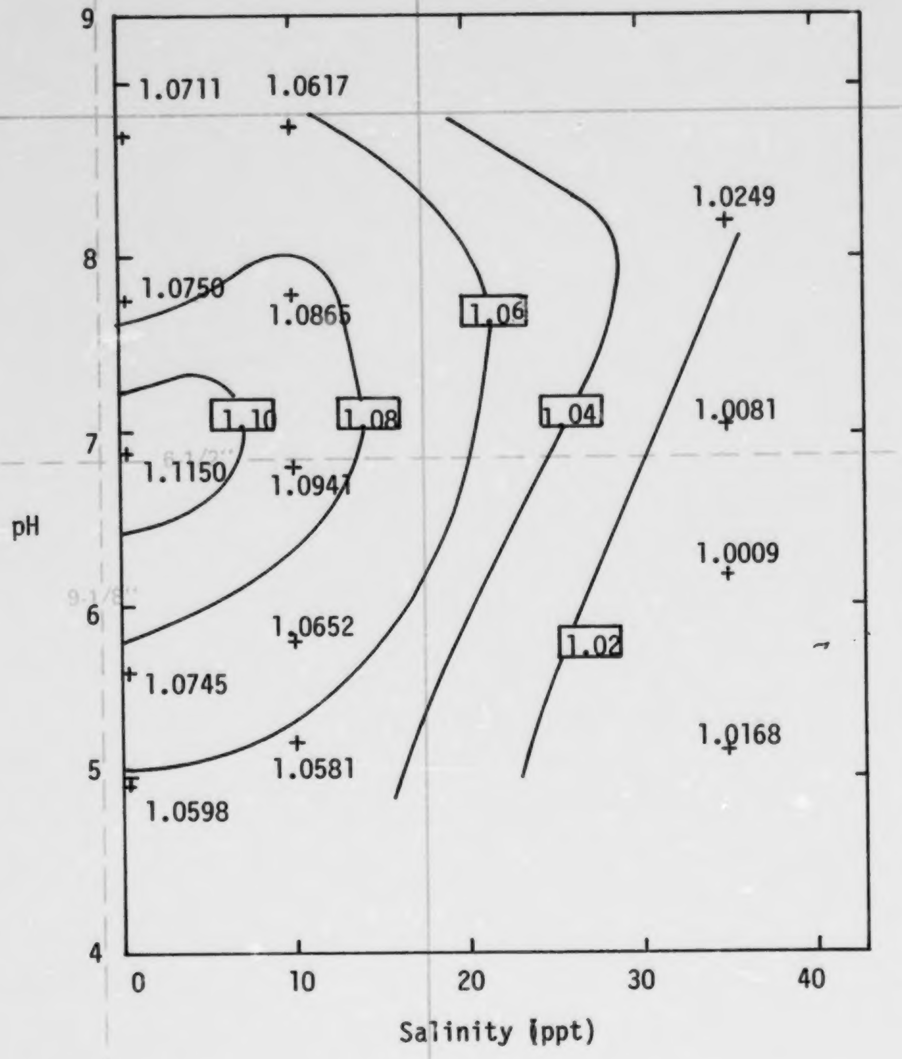

Figure 26. Temperature coefficient, 0, for the decomposition of methanol by acclimated sludge Iso-0 Lines at 5 - 22 ^{o}C.

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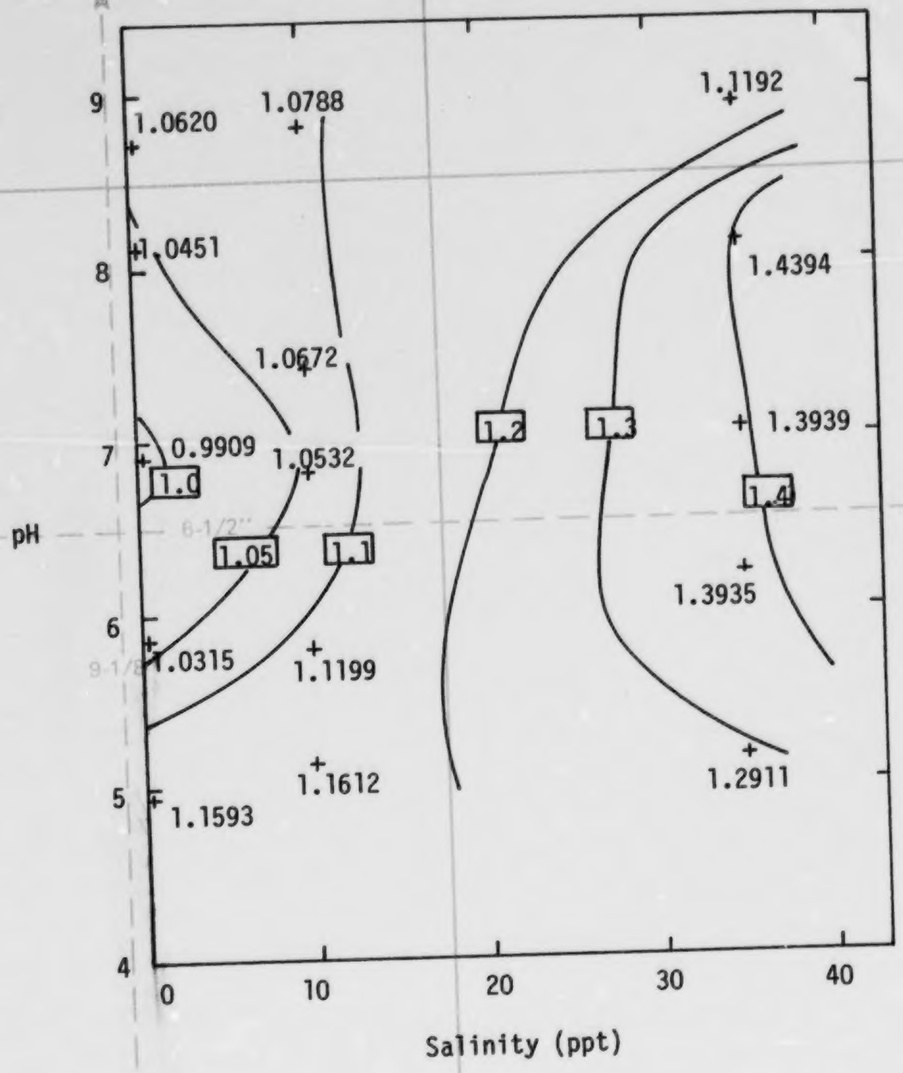

Figure 27. Temperature coefficient, 0, for the decomposition of methanol by acclimated sludge Iso-0 lines at 22 - 28 °C.

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Endogeneous Respiration —

The cell decay coefficient, k_d, showed a close relationship with the substrate removal rate coefficient, k, when tabulated according to the ranges of k (see Table 15 and Figure 28). The following relationship between k_d and k was established from the ln k_d vs. ln k graph (Figure 29) as follows:

$$k_d = 0.0115\ k^{0.634}, \qquad (71)$$

where k_d and k are based on the unit of hr^{-1}. The correlation coefficient between ln k_d and ln k was estimated to be 0.726.

TABLE 15. THE RELATIONSHIP BETWEEN SUBSTRATE REMOVAL RATE COEFFICIENT AND CELL DECAY COEFFICIENT FOR METHANOL-ACCLIMATED SLUDGE

Range of k (hr^{-1})	Number of observations	Average k (hr^{-1})	Average k_d (hr^{-1})
0.00 - 0.05	25	0.0298	0.001242
0.05 - 0.10	8	0.0693	0.002313
0.10 - 0.15	6	0.1332	0.003184
0.15 - 0.20	9	0.1791	0.004302
0.20 - 0.25	3	0.2375	0.000554
0.25 - 0.30	11	0.2783	0.005124

Nutrient Effects —

Reactors with nitrogen, phosphorus, and minerals and reactors without these nutrients all showed the same substrate removal rate ($k = 0.28\ hr^{-1}$) when the pH was buffered to pH = 6.9. Substrate removal patterns in two of these reactors are shown in Figure 30.

Methanol Decomposition in Natural Systems Without Chemical Aids --

When methanol-acclimated activated sludge of approximately 450 mg/l VSS was fed with 1,000 mg/l of methanol as TOC, the pH dropped from 7.6 to 7.1 in distilled water, from 8.6 to 7.5 in groundwater with an alkalinity of 115 mg/l, and from 8.4 to 8.2 in sea water with an alkalinity of 164 mg/l (see Figure 31). Since the pH did not change significantly, the most important factor affecting the decomposition rate was the initial pH of the water. The f-factor was 0.646 in distilled water, 0.315 in groundwater, and 0.112 in sea water (see Table 16). The f-factor of 0.315 in groundwater is only 47.3% of the value obtained from the f-factor diagram at 22°C (Figure 24). It is not certain what caused a greater pH change in groundwater than in distilled water and a much smaller substrate removal rate compared to the diagram.

Initial Lag Phase --

If the initial lag phase in methanol removal is a result of methanol decomposition of organic intermediates, the microorganisms will consume oxygen (as required by such an oxidation and by endogeneous respiration). Respiration rate measurements showed

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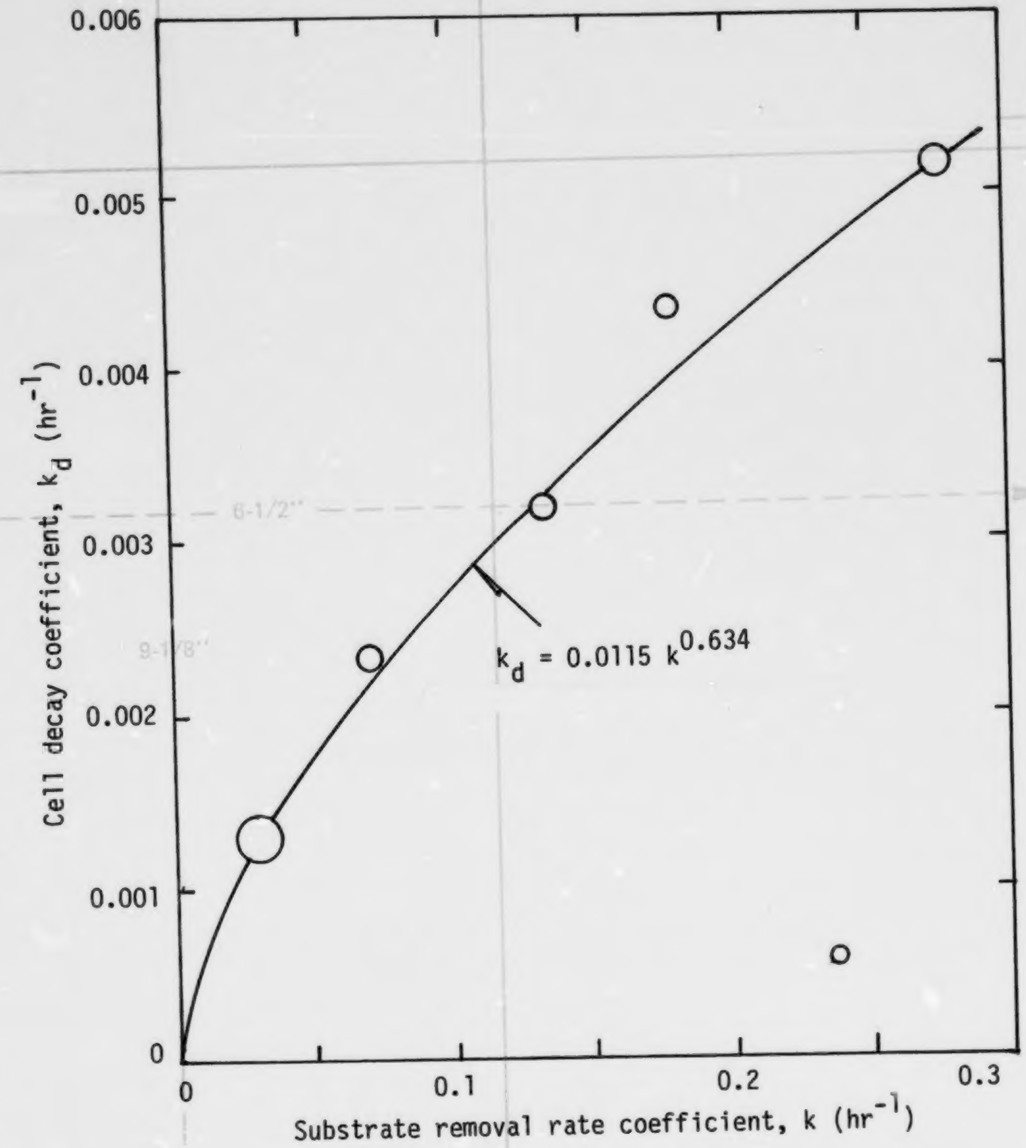

Figure 28. The relationship between the substrate removal rate coefficient and the cell decay coefficient for methanol acclimated sludge.

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FIG.28

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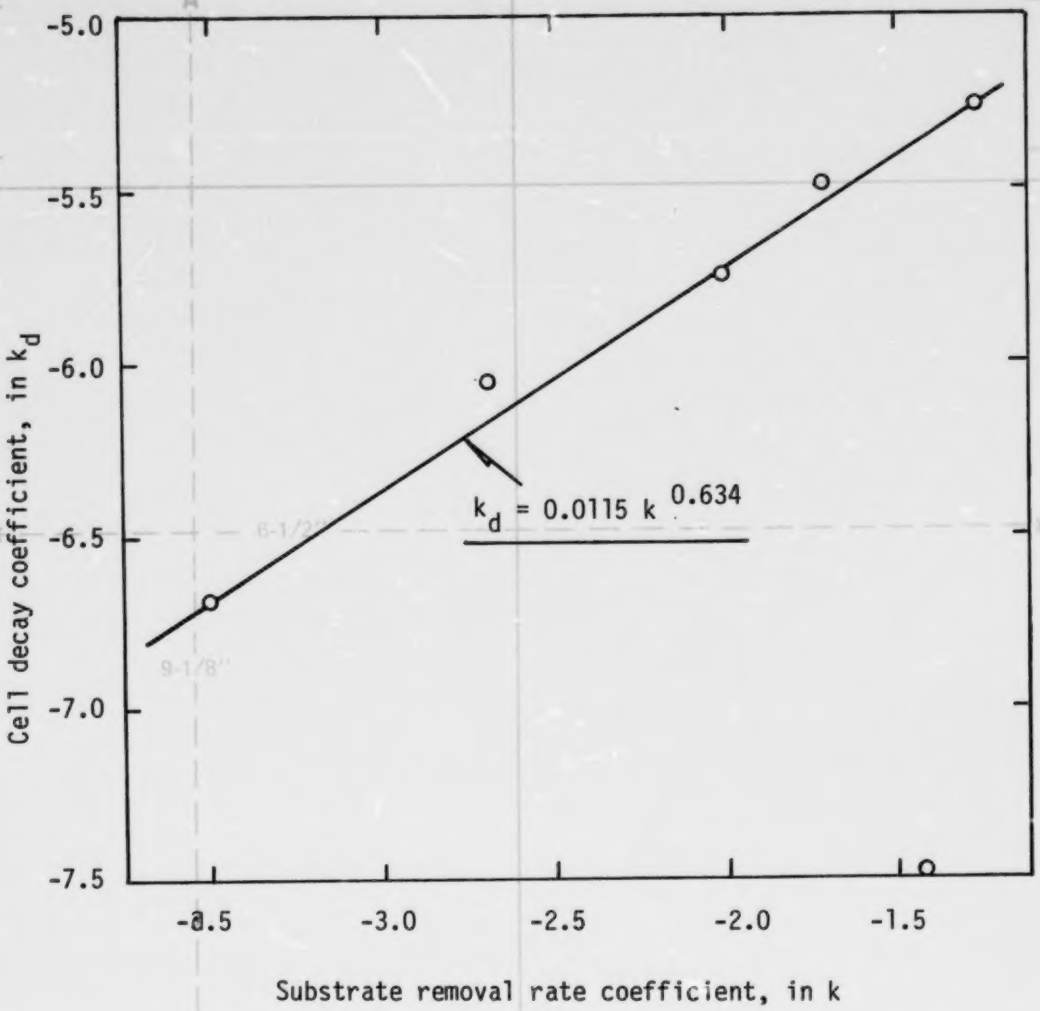

Figure 29. The relationship between the substrate removal rate coefficient and the cell decay coefficient for methanol acclimated sludge.

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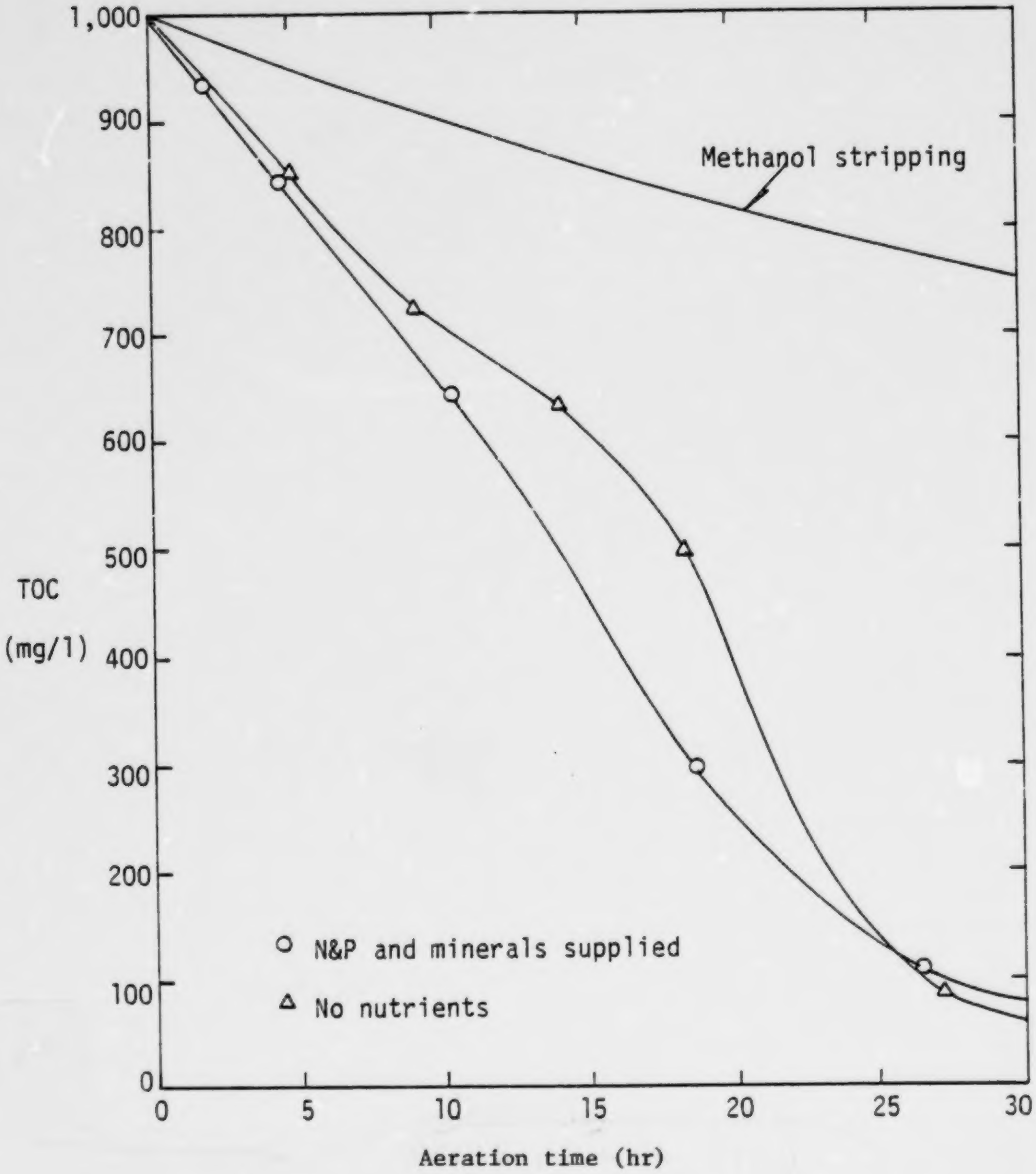

Figure 30. Nutrient effects on the decomposition of methanol by acclimated activated sludge.*

* Temperature = 22 ^{0}C, pH = 6.9, initial VSS = 450 mg/l

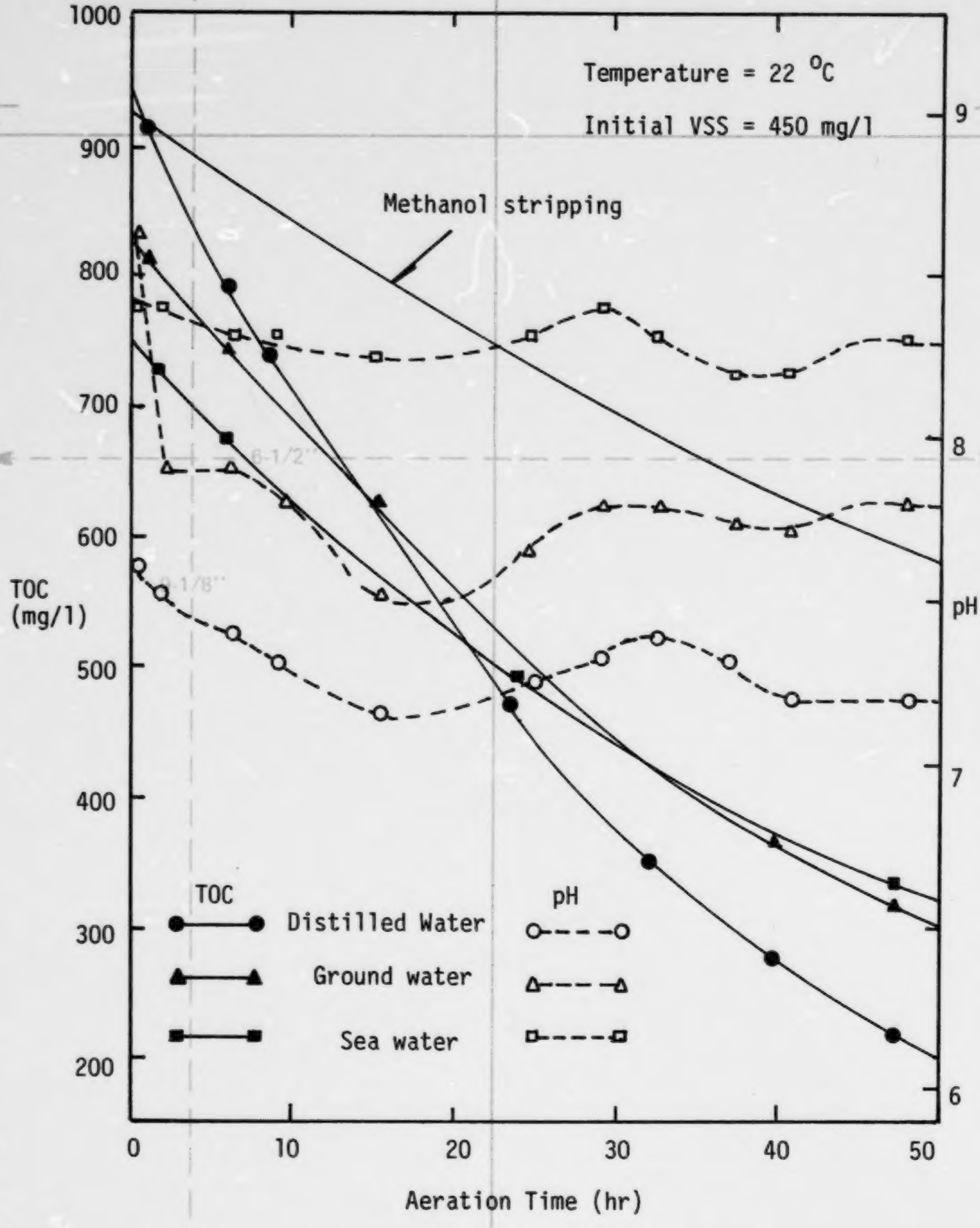

Figure 31. pH variations owing to the decomposition of methanol by acclimated activated sludge.

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that oxygen uptake was nil when there was no TOC removal by organisms at initial stages and that it increased as the TOC removal rate increased (see Figure 32). Thus, it is believed that the TOC removal rate is directly related to the bacterial activity.

Thirty-one of 66 tests showed an initial lag phase. The average duration of the initial lag phase in the 31 cases was 9 hours and the average aeration lag time was 6 hours (see Table 17). The longest lag phase was observed at pH = 5.6, salinity = 0 ppt,and temperature = 22°C and it lasted for about 37 hours, causing a 30-hour aeration lag time. The patterns of the inital lag phase are shown in Figure 33. It is not certain which factors were responsible for the lag phase and the duration of the lag phase. However, the duration of the lag phase generally appeared to decrease with increasing temperature, except for the above extreme case.

TABLE 16. METHANOL DECOMPOSITION BY ACCLIMATED ACTIVATED SLUDGE IN NATURAL SYSTEMS WITHOUT CHEMICAL AIDS[1]

Test waters / Parameters	Distilled water	Ground-water	Sea water
Initial pH	7.6	8.6	8.4
Alkalinity[2]	-	115	164
Hardness[2]	-	206	7,810
TDS (mg/l)	-	462	38,300
pH Range	7.1-7.6	7.5-8.6	8.2-8.4
Average pH	7.3	7.8	8.3
Average k (hr^{-1})	0.181	0.0884	0.0315
f	0.646	0.315	0.112
Efficiency of dilution water	0.813	0.473	1.0

[1] Temperature = 22 °C; k_{22^oC} = 0.2805 hr^{-1}.

[2] Expressed as mg/l $CaCO_3$

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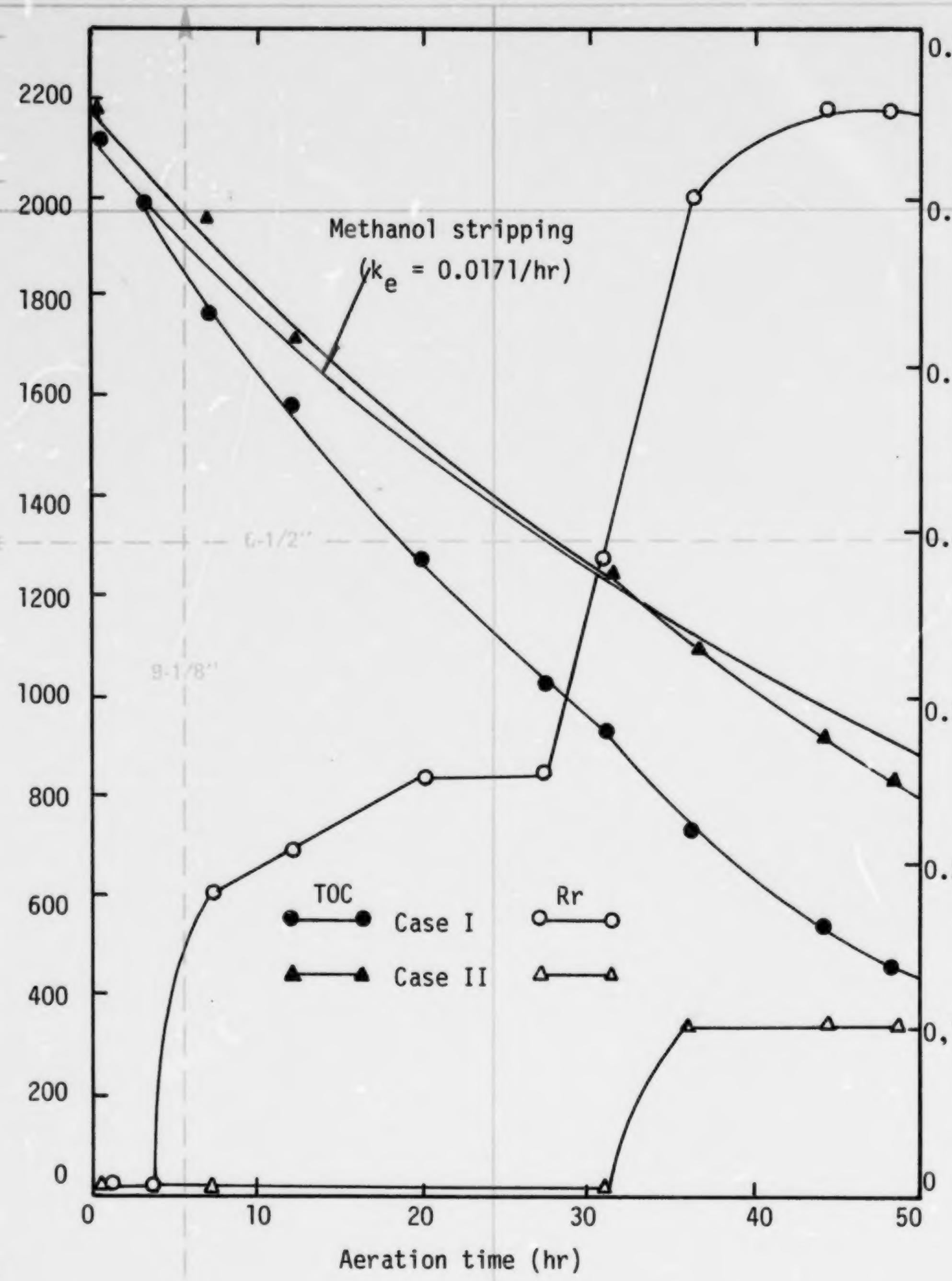

Figure 32. The relationship between substrate removal rate and oxygen uptake rate.

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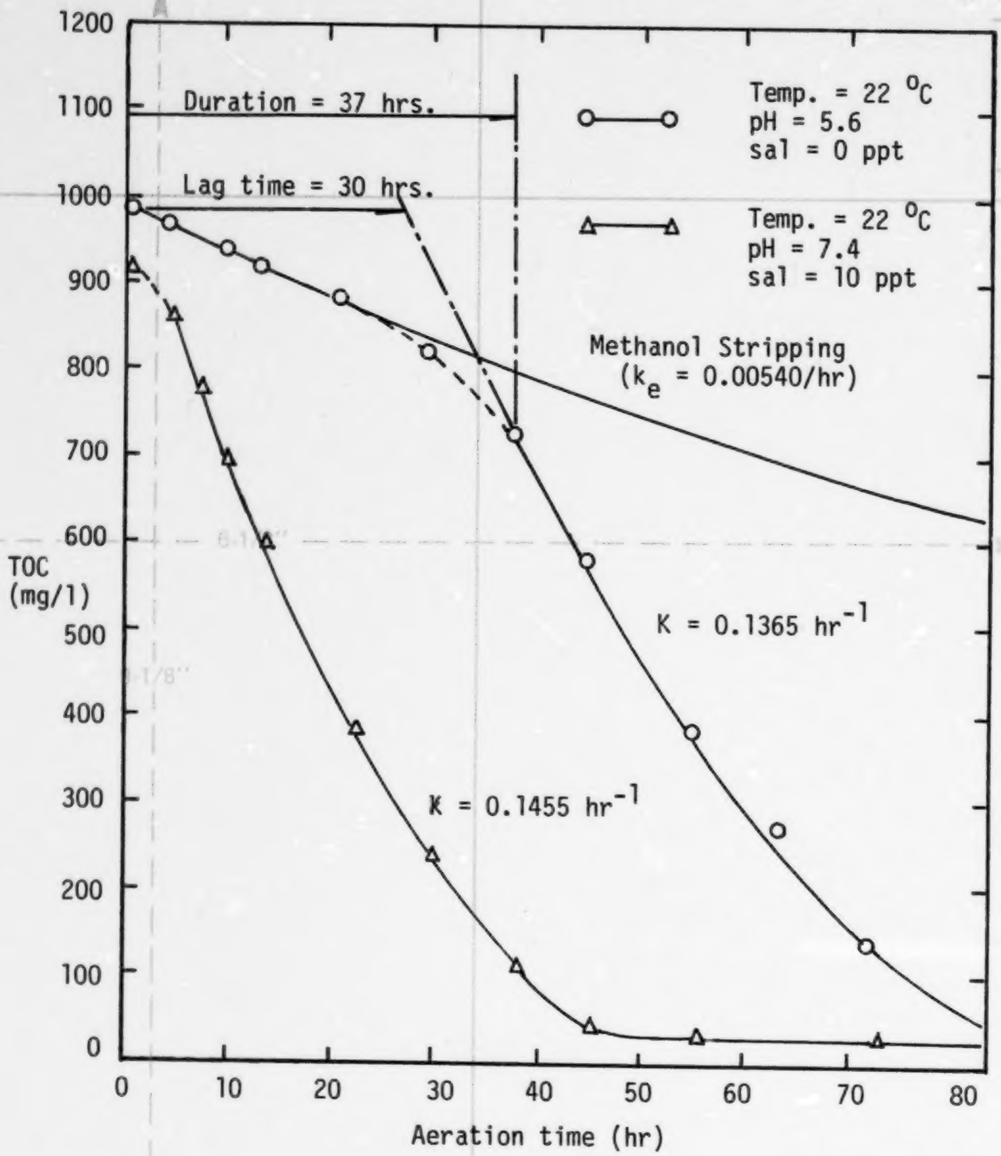

Figure 33. Initial lag phase in the decomposition of methanol by acclimated activated sludge.

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TABLE 17. INITIAL LAG PHASE IN METHANOL DECOMPOSITION BY ACCLIMATED ACTIVATED SLUDGE

Type of test	Temp. (°C)	Number of tests	Cases of lag phase	Average duration (hr)	Average lag time (hr)
Kinetic study	5	5	4	12.5	9.5
	22	2	-	-	-
	28	5	-	-	-
pH, salinity study	5	15	9	9	8
	22	15	7	13.5	8
	23	15	8	6	2.5
Nutrients study	22	5	-	-	-
Countermeasure application	24	3	3	7	4
	28	1	-	-	-
Total	-	66	31	9	6

Oxygen Requirement --

Complete oxidation of methanol requires 4 grams of oxygen per gram of carbon as indicated in the following equation:

$$\underset{(12)}{CH_3OH} + \underset{(48)}{\tfrac{3}{2} O_2} = CO_2 + 2\,H_2O \ . \qquad (72)$$

Complete oxidation of bacterial cell requires 2.67 grams of oxygen per gram of cell carbon (Equation 57). Thus, 1 gram of methanol carbon requires 1.33 grams (4.0 - 2.67 grams) of oxygen when it is utilized for cell synthesis.

One gram of methanol carbon removed by organisms results in 1.25 grams of organic fraction of cell production (a = 1.25), while theoretically, it will make 1.88 grams of organic solids if it is used entirely for cell synthesis. Therefore, 66.5% (1.25/1.88) is utilized for synthesis. Per gram of carbon, 66.5% of TOC decomposed by organisms will require 1.33 grams of oxygen and the remaining 33.5% of TOC removed by organisms will require 4 grams of oxygen. Thus, a' = (0.665 x 1.33) + (0.335 x 4) = 2.23 and b' = 1.42 k_d .

Then, the oxygen utilization rate (Equation 17) is expressed as:

$$Rr = -\,2.23\,\frac{dU}{dt} + 1.42\,k_d X \ , \qquad (73)$$

where $\frac{dU}{dt}$ and k_d are calculated in Equations 63, 64, and 71.

The oxygen requirement tests were conducted independently from the kinetic study tests on which Equation 73 is based. When 81 observations of oxygen uptake rate measurements were compared to the results from Equation 73, the mean difference between the observed and theoretical oxygen requirements was 0.317 mg/l O_2/hr ± 13.0 mg/l O_2/hr. Statistically, it was shown that this difference in oxygen utilization rate was not significant (see Appendix I,4). Therefore, it was concluded that Equation 73 could be used to predict the oxygen requirements.

In Figure 34, the theoretically evaluated values of a' and b' are justified through a linear graphical method. When Rr/X is plotted against $-(\Delta U/\Delta t)/X$, the plotted points should fall on a straight line, the slope and Y intercept of which are a' and b' respectively (Equation 17'). But, the experimental errors and variable transformations hardly allow any straight lines to be drawn. However, it is reasonable to assume that the probabilities of overmeasurement and undermeasurement of all the variables involved are the same. Therefore, the straight line must roughly divide the plotted points equally. Equation 73 fulfilled this requirement by dividing the observed points 38 to 43.

Through the above two justifications, it was concluded that $a' = 2.23$ (based on $a = .25$) and $b' = 1.42\ k_d$ (k_d is based on $k_d = 0.0115\ k^{0.634}$, Equation 71) provided a reliable estimation of oxygen demand.

p-Nitrophenol

Results of the p-nitrophenol screening tests were subjected to the same growth kinetic coefficient estimation procedures as were used for phenol and methanol. The estimated kinetic parameters are given in Table 18. The values for k and k_d are very similar to those for phenol, but the k_s concentration is substantially less, as is the value of a.

TABLE 18. ESTIMATED KINETIC PARAMETERS FOR PARA-NITROPHENOL[1]

Parameter	Expected value	Standard deviation	80% Confidence interval	X Range (mg/l)	S Range (mg/l)	N
K_s(mg/l)	42.39	88.14				
k(hr^{-1})	0.08509		$\geq$ 0.01785 $\leq$ -0.03075	20 to 240	up to 220	34
k_d(hr^{-1})	0.004058	0.003963				
a	0.4300	0.1125				

[1] Temp. = 20°C

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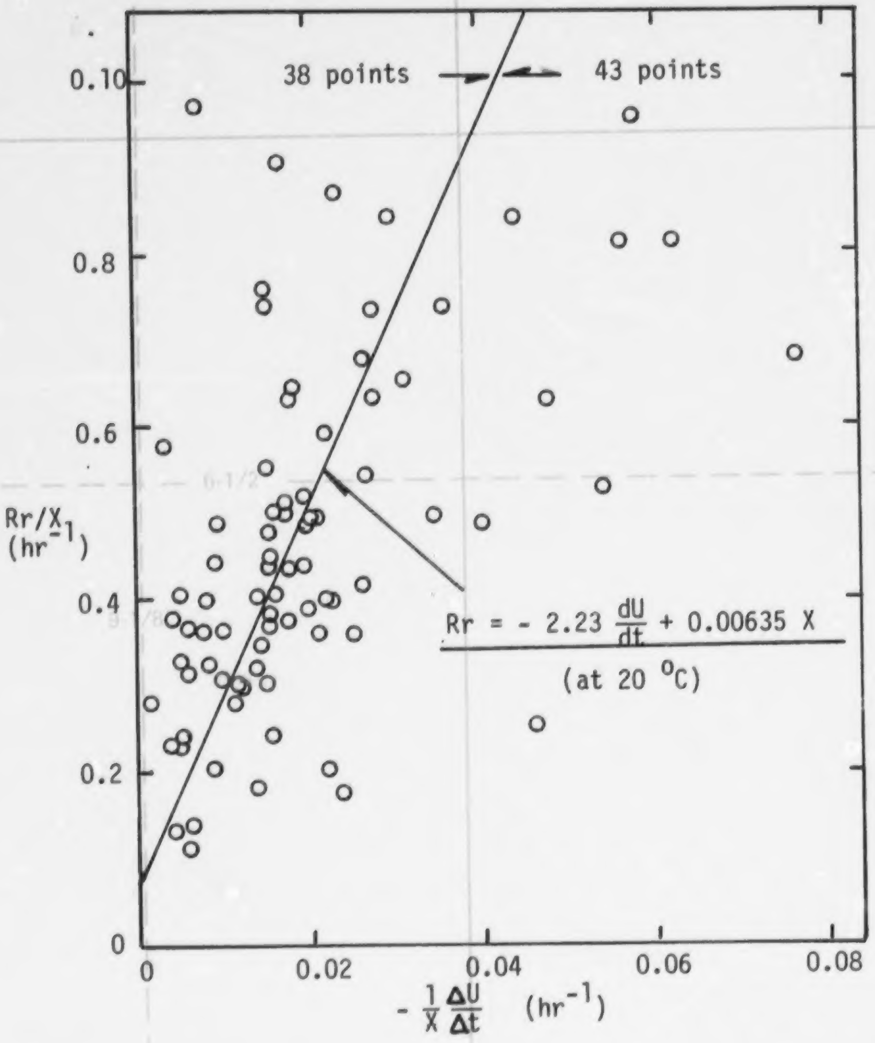

Figure 34. Comparison of theoretical and observed oxygen uptake rates in methanol decomposition.

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SECTION 9

SIMULATED SPILL TESTS

To determine toxicity of the test compounds in natural systems and to test deployment methods for the biological countermeasures, a number of model systems were established. Fifteen aquaria, of 57-liter capacity each, were established with sediments, plants, and crustacea to simulate a portion of a slow-moving stream or a pond. These aquaria received a continual flow of aged tap water initially, and, later, groundwater, with a residence time of about 2 days. Because these aquaria could be used, cleaned, and reestablished rather quickly, they were used initially to determine the response of natural systems to spillage of the contract list of hazardous materials. Total system productivity was monitored to determine this response. Then, hazardous materials were spilled into the aquaria to test the *in situ* and portable treatment system countermeasure techniques. Ten small pond ecosystems were also established. Each was 1.8 meters in diameter, contained 800 liters of water, was stirred continuously by a pump, and was furnished with sediments and plants like the aquaria. These systems were used to simulate a portion of a pond and their size diminished some of the wall effects characteristic of the aquaria.

The results of the aquaria and of the pond tests were used to develop experimental methods for large-scale biological countermeasure tests in a 30.5-m diameter, 3.0-m deep model lake. Spills of hazardous materials were made in the tank and the *in situ* countermeasure techniques used to mitigate the material were evaluated. Flowing-system tests were carried out in a model river, which had been used previously for radioactive material transport studies. The model river is 61 meters in length and consists of two parallel channels, each equipped with flow and water level regulation devices and established with sediments, plants, and small aquatic organisms. The experiments carried out in the model river system are described in Section 11 along with the countermeasure application techniques.

The aquaria and ponds were located in temperature-regulated rooms with controlled fluorescent lighting, while the model river and model lake were located outside.

While the primary purpose of these studies was to investigate the feasibility of using a biological countermeasure in a near real-life system, secondary objectives were: (1) to examine hazardous material removal rates as a function of bacterial culture, amounts of bacterial mass (specifically, the ratio of mass of hazardous material spilled to the bacterial mass applied), the addition of nutrient salts, the method of bacteria application, and the effects of dilution in flowing systems and (2) to examine the effects of the biological countermeasures on the quality and ecosystems of the receiving water.

AQUARIA TESTS

Rationale

As mentioned above, the aquaria tests were designed to provide initial experience in the use of biological countermeasures for spilled hazardous materials in ecosystems, as well as to show the effects of the spills. It was assumed that removal of spilled materials in the aquaria could be monitored as it had been in the treatment reactors. Therefore, the general rationale of the experiments was to "spill" one of the test chemicals, follow its removal over time through sampling and analysis, and determine the effects of the countermeasure on the system. Rates of removal, effects of the ratio of material mass spilled to bacterial mass added on the removal rates, and effects of nutrient additions were investigated.

The basic measurement parameter, removal rate of the spilled material, was calculated in the following way.

Because the aquaria used in these studies were continuously supplied with fresh water and were mechanically mixed by stirrers, the aquaria could be treated as continuous-flow, stirred reactors. The concentrations resulting from spills into such systems may be described by the following equation:

$$S = S_o e^{(\frac{1}{\theta} + k)\, t} , \qquad (74)$$

Where:
S = concentration of spilled material at time t after spillage (M/L^3),
S_o = initial concentration of spilled material = M/V (M/L^3),
θ = hydraulic residence time = V/Q(T),
k = biological decomposition rate ($M/(TM)$),
t = time (T),
V = system volume (L^3),
Q = flow into or out of system (L^3/T), and
M = mass spilled (M).

Thus, in these spill control tests, it was desired that k be calculated by measuring S at various times during the test, determining the overall removal rate of the spilled material, and then solving for k knowing the hydraulic dilution rate, $1/\theta$.

Procedures

Apparatus --

The 57-liter capacity aquaria used were 60 cm long, 31 cm wide, and 29 cm deep. The water level was maintained so that with sediments in the bottom, the working water volume was about 45 liters. Aged tap water and, later, groundwater were metered into each aquarium through tygon tubing constricted by a screw clamp valve. Flow rates were measured twice daily and adjusted when necessary (because of their tendency to change over time). At the outlet end of each aquarium, a constant level siphon was used, which had to be serviced periodically to remove attached algae and bacteria. The water in each aquarium was gently stirred by high torque stirrers equipped with a stirring rod to which was attached a smaller cylinder perforated with holes. Dye tests showed that at most, complete mixing occurred within a few minutes.

Dissolved oxygen measurements were made with a Precision Scientific Co. probe and temperature was measured by a thermocouple on the probe or by a mercury thermometer.

The aquaria were illuminated by banks of 40-watt fluorescent lights placed directly overhead and were put on a 12-hour "on", 12-hour "off" cycle. Light intensities were in the range of 300 to 500 foot-candles at the level of the plants in the aquaria.

Nine of the aquaria (Aquaria 1 through 9) were operated individually, that is, water was metered into and drained from each aquarium . Six of the aquaria (Aquaria 10 through 15) were operated in series. Water was metered into Aquarium 15 and was conveyed to Aquarium 14, then Aquarium 13, and so forth through siphons; water was drained from Aquarium 10. The configuration of these aquaria in the laboratory is shown in Figure 35 and the water depths and volumes of each aquarium are given in Table 19.

TABLE 19. WATER DEPTHS AND VOLUMES OF AQUARIA

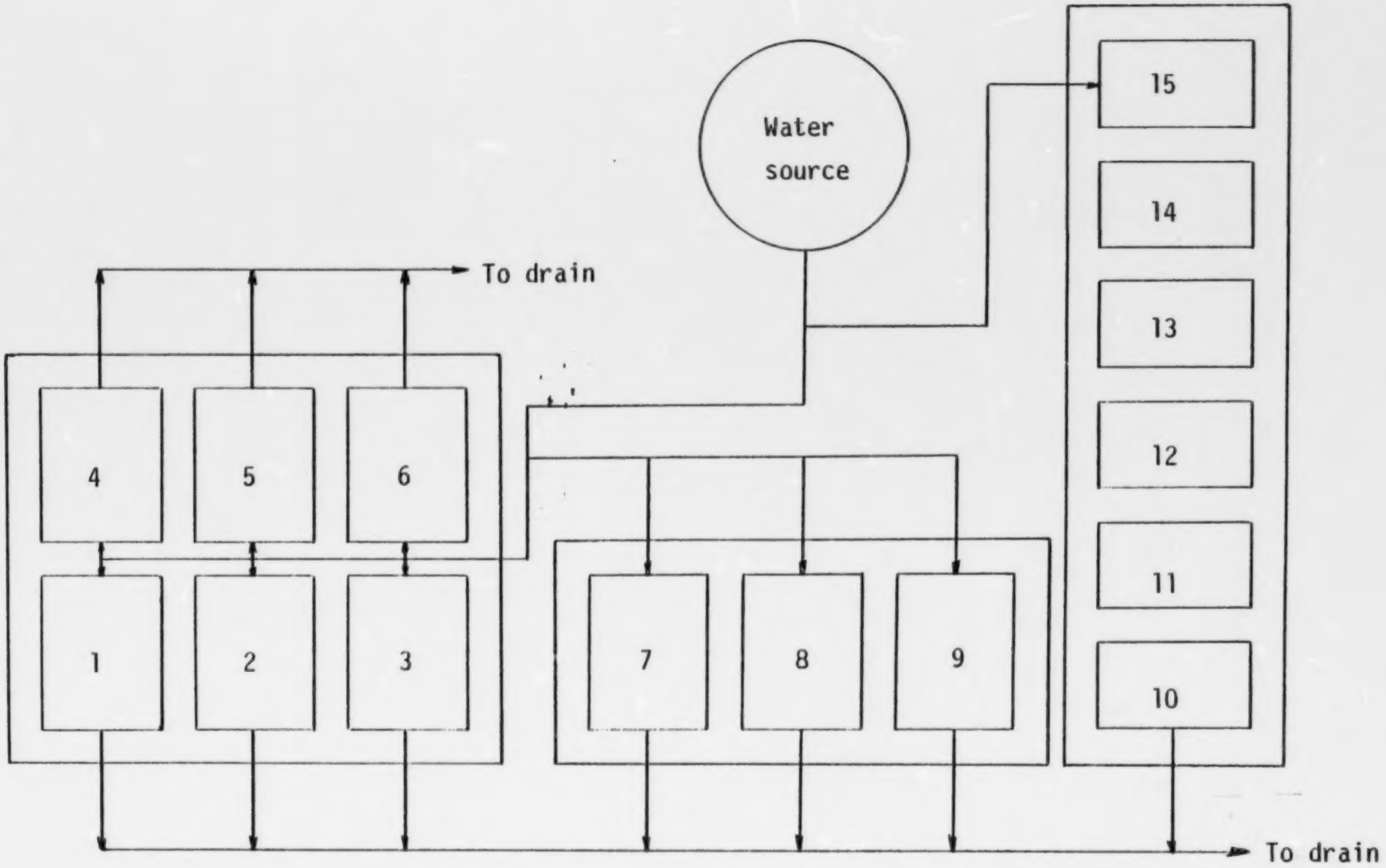
Aquarium number	Water depth (cm)	Water volume (ℓ)	Aquarium number	Water depth (cm)	Water volume (ℓ)
1	25.4	46.3	9	25.4	46.3
2	24.8	45.2	10	22.2	40.6
3	23.8	43.4	11	22.5	41.2
4	23.8	43.4	12	24.8	45.2
5	24.8	45.2	13	24.1	44.0
6	23.8	43.4	14	23.5	42.9
7	24.4	44.6	15	24.1	44.0
8	23.8	43.4			

Experimental Procedures --

The general procedure for all tests conducted was to permit the aquaria ecosystems to stabilize for several weeks before a test was begun. The systems were considered to be stable when the production and respiration values and ratios were relatively constant.

After the material to be spilled was selected, the amounts to be spilled and the amounts of acclimated or unacclimated bacteria to be added were determined. Flow rates, thus residence times, were set and a sampling schedule was established.

A culture of bacteria was fed the test chemical in a reactor for several weeks prior to the spill tests. Just prior to the spill tests, the culture was allowed to settle, the supernatent was decanted, and a measured volume (about 10ℓ) of the sludge was readied for addition to the aquaria. The TSS and VSS content of the bacteria culture were measured after settling.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To drain
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14
13
12
11
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6
1
2
3
7
8
9
To drain

Figure 35. Configuration of aquaria in laboratory.

Just prior to spillage, a volume of water equal to the bacterial culture volume was removed from the aquarium to receive the bacteria so that the aquarium volume returned to a desired level upon addition of the culture. This procedure was not done for test chemical additions since the volume added was usually only a few milliliters.

Spilling was accomplished by simply pouring the test chemical or bacterial culture into the appropriate aquarium near the stirrer to insure mixing.

Generally, a water sample was taken prior to spillage and then after spillage with decreasing frequency over a period of a few days to several months. These samples were routinely analyzed for TOC, total oxygen demand (TOD), pH, TSS, VSS, and the compound spilled (gas chromatography). Occasionally, plate counts were made.

Daily dissolved oxygen and temperature measurements were made immediately after the lights came on and after they were turned off so that production and respiration values could be calculated using the three-point method. Hourly samples for dissolved oxygen were taken on several occasions to verify that the three-point method could be applied.

Data Analysis --

After each test, the TOC or gas chromatograph results of sample analyses were plotted on semi-logarithmic paper to permit calculation of the slope of the curve. From this slope value, the hydraulic dilution rate was subtracted to obtain the biological decomposition rate, which was then related to the experimental conditions imposed. VSS and plate count data were plotted on arithmetic paper to follow the net growth of the bacteria added over the period of the experiment.

The dissolved oxygen and temperature data were used to calculate the production and respiration rates and their ratios. These data were then plotted with time to show the effects of the material spilled and of the countermeasure on the ecosystem.

Results

Two hazardous materials were used in the aquaria spill tests -- phenol and methanol. The results of the tests using these two compounds are described below.

Phenol --

A total of six sets of experiments were conducted in the aquaria using phenol.

Removal test No. 1 -- The first of these tests was performed during the period from March 17 to May 1, 1974 with spill monitoring occurring on April 10-13, 1974. Five aquaria were used in the initial experiment (Aquaria 1,2,3,4, and 6); three of these aquaria (Aquaria 1, 2, and 3) contained the rooted plant Vallisneria and the other two (Aquaria 4 and 6) contained only phytoplankton. One aquarium in each set was used as a control (Aquaria 1 and 6) and no phenol was spilled into these aquaria. Also in each set, one aquarium received phenol (theoretical initial concentration of 95 mg/l) but no bacteria (Aquaria 2 and 4). In the aquaria with the Vallisneria, one aquarium received phenol plus bacteria (Aquarium 3).

Following the "spill" of phenol into the aquaria, the concentrations of phenol and bacteria were monitored using gas chromatography, TOC, TOD, and VSS analysis and plate counts. The results of the gas chromatography and bacterial concentration analyses are presented in Figures 36 and 37. The results for Aquarium 2 given in Figure 36 show a decrease in phenol due strictly to washout of phenol by water flowing through the system. Note that the VSS concentrations were very small.

In contrast, the decrease in phenol concentration in Aquarium 3, which received phenol and bacteria, was very rapid after the first 24 hours. Note in the lower portion of Figure 37 that the VSS concentration representing the bacterial mass was high initially and dropped off to a rather stable concentration of about 12 mg/ℓ. The bacteria added to this aquarium settled rapidly onto the sediments and grew there for several days before disappearing through self-oxidation. Also given in the lower portion of Figure 37 are plate count concentrations of bacteria that are able to use phenol as a substrate.

The observed rates of decay of phenol in these aquaria are given in Table 20. When the dilution rates are subtracted from the observed decay rates, one may obtain the rate of biological decay of phenol. In the two aquaria that received only phenol (Aquaria 2 and 4), the biological decay rate was larger than the dilution rate. The net effect of adding bacteria was to reduce phenol concentrations to less than detectable levels within three days.

TABLE 20. SIMULATED SPILL OF PHENOL IN AQUARIA, REMOVAL TEST NO. 1
(APRIL 1970 to MAY 3, 1974)

			Disappearance rates (d^{-1})			Environmental Conditions		
Aquarium number	Conditions	Initial phenol conc.	Obsd. decay rate	Dilution rate	Biol. decay rate	Avg. pH	Avg. DO (mg/1)	Avg. temp. (°C)
1	Control	0	-	0.473	-	8.5	9.0 (6.5)[2]	22.5
2	Phenol only	90.8	0.624	0.479	0.145	8.3	8.5 (3.0)	22.6
3	Phenol + bact.[1]	94.5	1.122	0.505	0.617	7.4	1. (1.)	22.7
4	Phenol only	94.5	0.733	0.505	0.228	8.1	8.4 (1.7)	22.5
6	Control	0	-	0.505	-	8.7	9.5 (7.0)	22.2

[1] Phenol/VSS ratio = 0.98 mg/mg.

[2] Lowest DO value.

Dissolved oxygen values recorded during these tests are shown in Figure 38. As expected, the control aquarium showed little change in dissolved oxygen, but the aquarium receiving phenol and bacteria exhibited an immediate drop to near zero dissolved oxygen. After a one-day delay, the dissolved oxygen in the aquarium

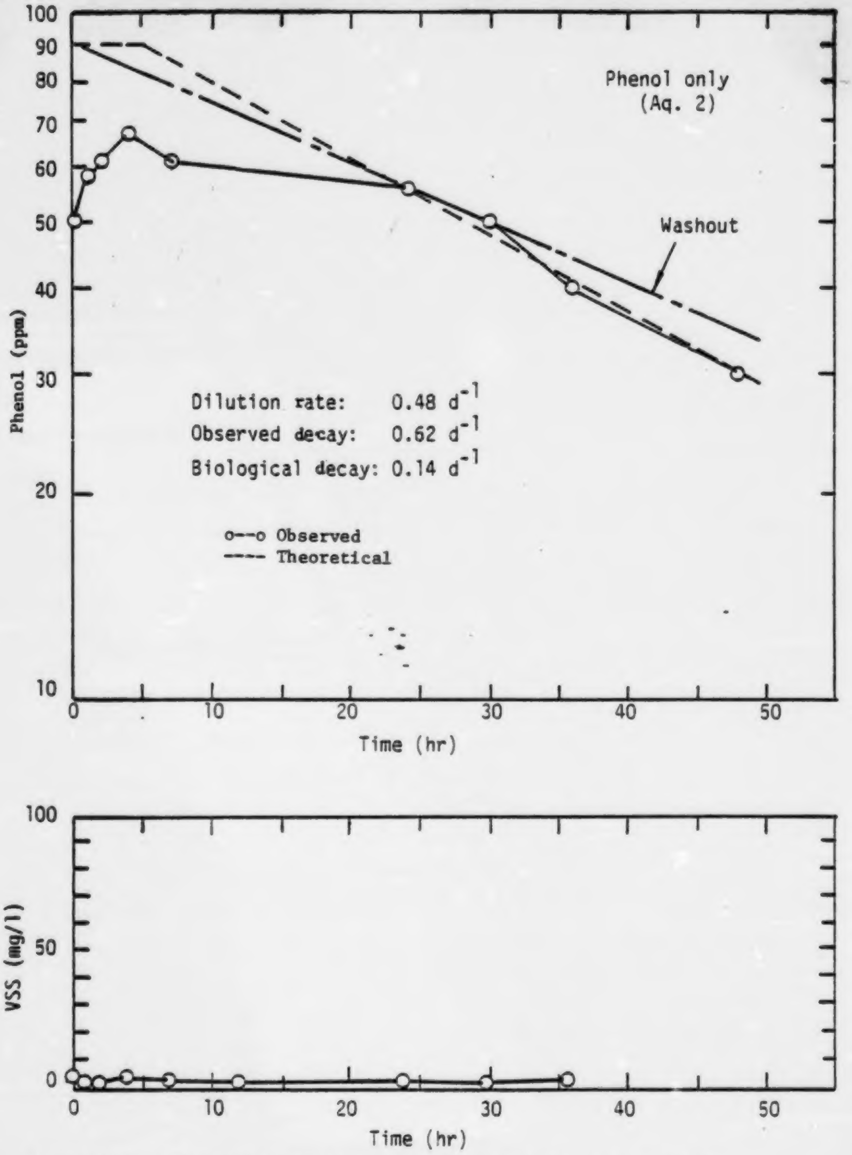

Figure 36. Analytical results of removal test No. 1, Aquarium 2 (phenol only).

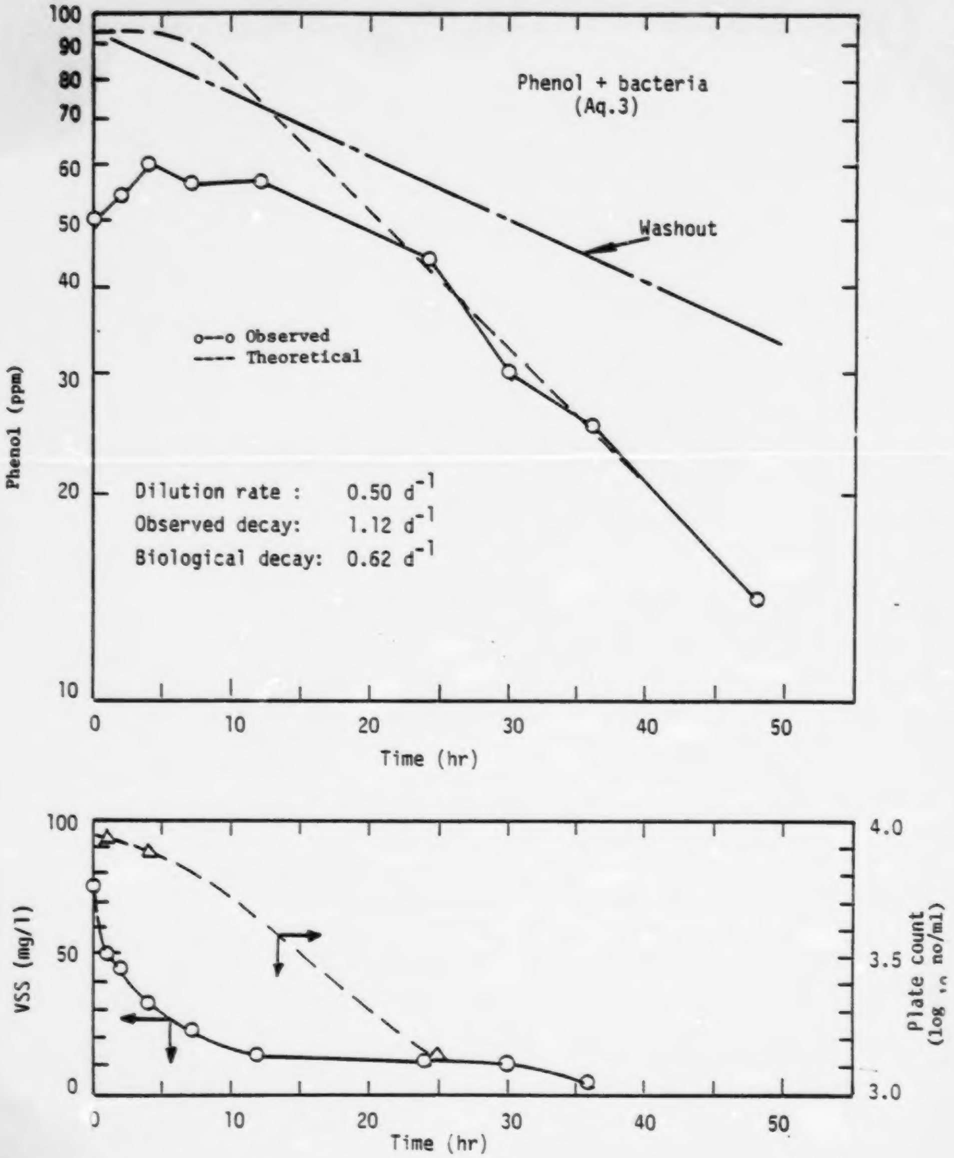

Figure 37. Analytical results of removal test No. 1, Aquarium 3 (phenol + bacteria).

receiving no phenol dropped, apparently as a result of bacterial growth and phenol decomposition. Results of the production and respiration analyses are given in Figures 38, 39, and 40. The gross production (P_G) and total respiration (R_T) in the control aquarium (Aquarium 1) are given in Figure 39. As indicated in the figure, production and respiration averaged about 7 mg/l/d. There was a slight increase in P_G and R_T from day 0 to day 10, which occurred as the aquarium was changed from a static to a flow-through system. Stabilization of this aquarium following the change in the nature of the system is also shown in the ratio of gross production to total respiration, which is given in the lower portion of Figure 38.

Once phenol was spilled, the ecosystems in the aquaria responded rapidly. In Aquarium 2, which received only phenol, there was a two-day delay in response to the spill as shown in Figure 40. On the second day following the spill, gross production of the system dropped to near zero, while total respiration increased to over 9 mg/l/d. However, respiration suddenly dropped to near zero, but five days after the spill, production and respiration began to recover to values higher than pre-spill levels. Some cycling of production and respiration levels is evident between days 20 and 40 in Figure 40. These overall changes are also reflected in the ratio of production to respiration, which is given in the lower portion of Figure 40.

A similar response was given by the ecosystem in Aquarium 4; however, because this aquarium received bacteria and phenol, the response was more immediate. Within a few hours following the addition of bacteria, the dissolved oxygen dropped to zero in the aquarium. The gross production dropped to near zero and respiration increased rapidly for a day then dropped to just under 5 mg/l/d. By the fourth day after the phenol spill, the ecosystem was recovering and production and respiration levels far exceeding the prespill levels were measured as shown in Figure 41. Maximum production and respiration values of about 19 mg/l/d were reached. These overall changes are also reflected in the ratio of production to respiration, which is given in the lower portion of Figure 41.

It is obvious that the ecosystems in these aquaria responded dramatically to the phenol spill. The aquaria were monitored almost continuously following the spill and they returned to essentially pre-spill conditions.

The bacterial concentrations present were measured as volatile suspended solids (VSS) or were determined from a plate count (Figures 35 and 36). In Aquarium 2, essentially background levels of 3 to 4 mg/l VSS were found, whereas much higher levels were found in Aquarium 3, to which bacteria had been added. However, these added bacteria settled to the bottom of the aquarium and remained there as a white, fluffy mass until long after the phenol had been completely removed. After 15 to 20 days, this bacterial layer disappeared.

Removal test No.2 --A second test was performed during the period April 19 to June 3, 1974 to confirm the results of the first test. Using Aquaria 7, 8, and 9, enough phenol was spilled to produce an initial concentration of approximately 100 mg/l in Aquaria 8 and 9, while Aquarium 7 was kept as a control. The results of this test are given in Table 21.

The effects of phenol addition on dissolved oxygen levels and on production and respiration were essentially the same as in the first test. The only difference was that the production values in Aquarium 8, receiving only phenol, did not drop as much as in Aquarium 2 in the earlier test.

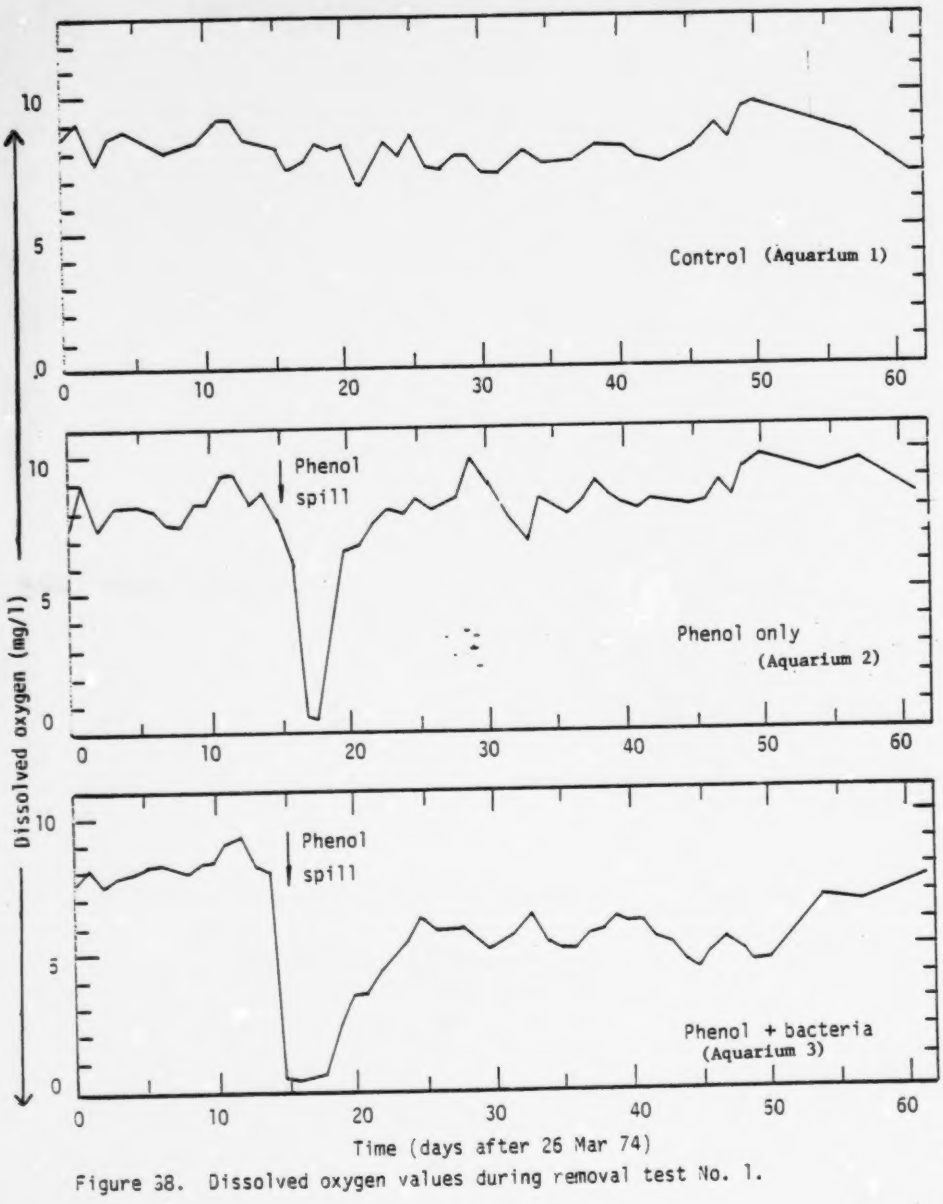

Figure 38. Dissolved oxygen values during removal test No. 1.

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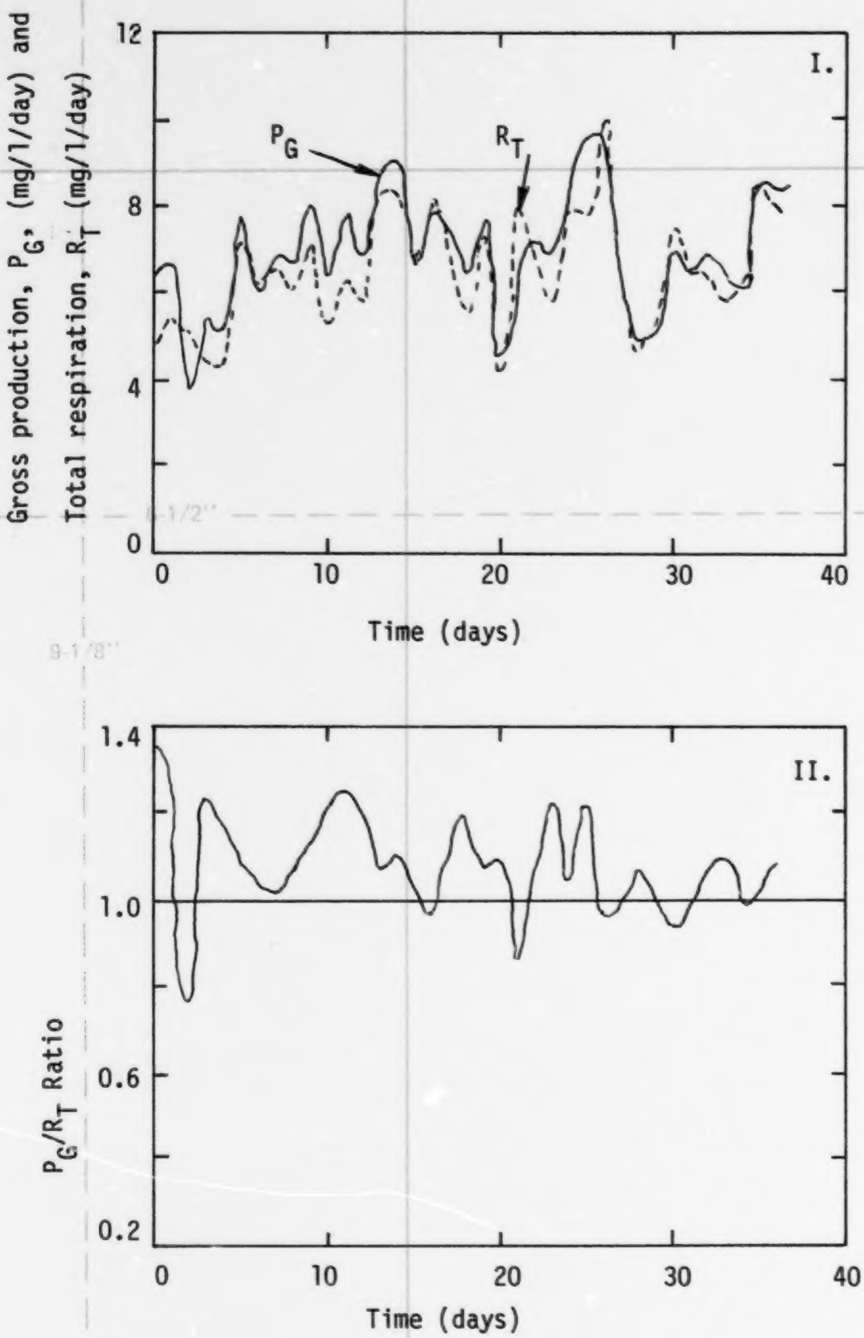

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Figure 39. Gross production (I) and total respiration (II) in aquarium I (control) Removal test No. 1.

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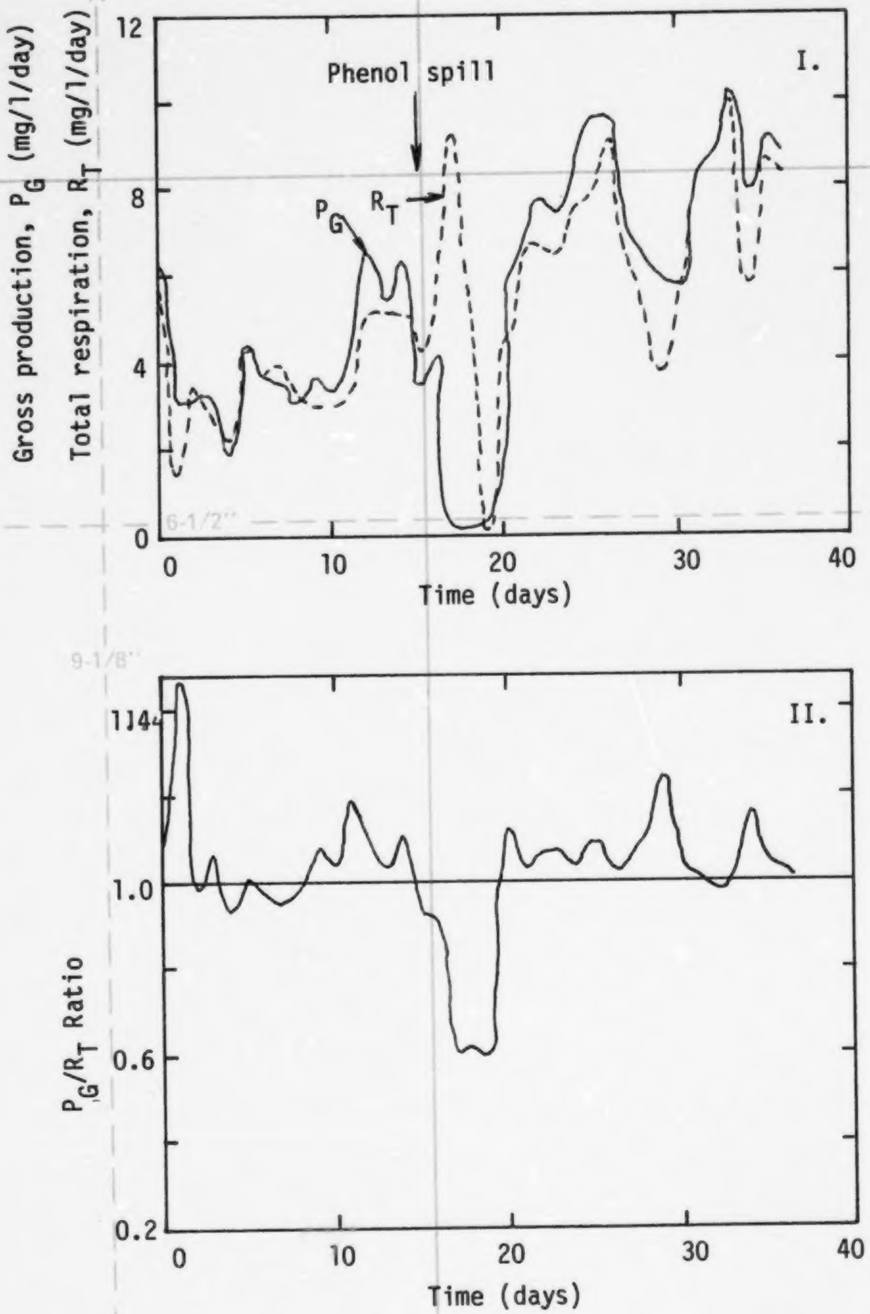

Figure 40. Gross production (I) and total respiration (II) in aquarium 2 (phenol) removal test No. 1.

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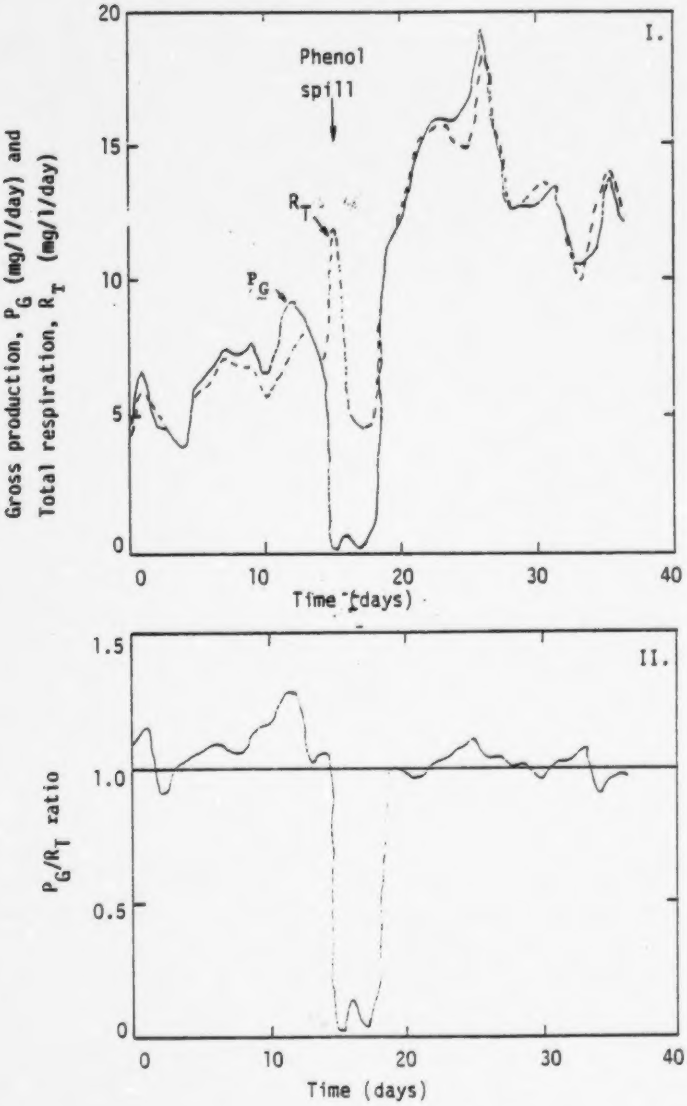

Figure 41. Gross production (I) and total respiration (II) in Aquarium 3 (phenol + bacteria). Removal test No. 1.

TABLE 21. SIMULATED SPILL OF PHENOL IN AQUARIA, REMOVAL TEST NO. 2 (APRIL 19 to MAY 3, 1974)

			Disappearance rates (d^{-1})			Environmental Conditions		
Aquarium Number	Conditions	Initial phenol conc.	Obsd. decay rate	Dilution rate	Biol. decay rate	Avg. pH	Avg. DO (mg/l)	Avg. temp. (°C)
7	Control	0	-	0.485	- (7.5)[2]	8.2	9.1	20.5
8	Phenol	92.2	1.83	0.51	1.32 (0.0)	8.2	8.0	20.6
9	Phenol + bact.	86.5	3.30	0.49	2.81	6.7	1.	20.9

[1] Phenol/VSS ratio = 1.1 mg/mg.

[2] Lowest DO value.

Effect of phenol/VSS mass ratio test -- To determine how much bacterial mass must be added to a known mass of spilled chemical, a test was carried out during May 1974. Phenol and bacteria were added in such proportions that their ratio ranged from 12.9 to more than 4,300 mg/mg. Also tested was a bacterial culture, developed by the microbiology group, that was acclimated to phenol and that would stay in suspension.

The initial phenol concentration in each of the three aquaria (Aquaria 4, 5, and 6) was 485 ppm. Aquaria 4 received a phenol and bacterial culture developed from activated sludge, while Aquaria 5 and 6 received phenol and two different volumes of an unacclimated bacterial culture developed by the microbiology group.

The results of these tests are given in Table 22 and show the importance of the phenol/VSS mass ratio. At the lowest mass ratio, the biological decay rate was $3.1\ d^{-1}$, while it was less than 0.4^{-1} at higher ratios. It should be noted that, in each case, a 48-hour time lag was observed before significant decomposition of phenol occurred.

Because adding large amounts of bacteria can lower dissolved oxygen concentrations to zero, the removal rate can be inhibited. Thus, one must strike a compromise between the amount of bacteria added and the risk of lowering dissolved oxygen to inhibiting levels. In this particular experiment, Aquarium 4 was aerated, dissolved oxygen levels remained above 5.0 mg/l, and a very high removal rate was observed. Aquaria 5 and 6 were not aerated, dissolved oxygen levels dropped to near zero, and lower removal rates were observed. When the results for Aquarium 3 in the previous experiment (Table 20) are compared with those of Aquaria 5 and 6, it is still apparent that a low phenol/VSS mass ratio is desirable, but aeration can greatly enhance the removal rate, as in the case presented here, by a factor of 5.

Nutrient Addition Test -- The next test was performed to examine the importance of nutrient additives to the biological countermeasure. In the treatability studies, mineral salts had to be added to provide the necessary amounts of nitrogen, phosphorus, and other nutrients for bacterial growth. The nutrient addition test was carried out in January 1975 and involved three test conditions: (1) Aquarium 1 received phenol only (500 ppm), (2) Aquarium 2 received phenol (500 ppm) plus bacteria, and

(3) Aquarium 3 received phenol (500 ppm) plus bacteria and $NaHCO_3$, KH_2PO_4, and $(NH_4)_2SO_4$ in amounts sufficient to provide the concentrations of nitrogen and phosphorus used in the treatability studies.

The results of these tests (Table 23) show that nutrient additions produced a two-fold increase in the decay rate as compared to Aquarium 2, even though the dissolved oxygen level fell to zero near the end of the test. The importance of nutrients was reflected in the VSS concentrations, which are indicative of bacterial concentrations. In Aquarium 1, the VSS concentration never exceeded 12 mg/l. In Aquarium 2, which received bacteria but no nutrients, the VSS dropped from an initial concentration of 214 mg/l to less than 10 mg/l within six hours and stayed below 6 mg/l for the rest of the test. In contrast, the VSS concentration in Aquarium 3 dropped initially (as in Aquarium 2) but then rose after 10 hours following the spill to 38 mg/l before decreasing again.

TABLE 22. EFFECTS OF PHENOL/VSS MASS RATIO ON SPILL REMOVAL RATE (MAY 1974)

		Disappearance rates (d^{-1})			Environmental Conditions		
Aquarium number	Phenol/ VSS mass ratio[1] (mg/mg)	Obs. Decay rate[2]	Dilution rate	Biol. decay rate	Avg. temp. (°C)	Avg. D. O. (mg/l)	Avg. pH
4[3]	12.9[4]	3.58	0.5	3.1	21.5	8.0 (7.5)[7]	7.3
5	43.7[6]	0.88	0.5	0.38	21.5	5.0 (0.0)[5]	7.4
6	4367.4[6]	0.76	0.5	0.26	22.0	5.0 (0.0)[5]	7.5

[1] Phenol added to each aquarium was 21,400 mg.

[2] Rates observed over 48-hour time lag.

[3] Aerated.

[4] Activated sludge-adapted bacteria.

[5] Lowest D.O. value.

[6] Laboratory culture of adapted bacteria.

TABLE 23. EFFECTS OF NUTRIENT (NITROGEN AND PHOSPHORUS) ADDITION WITH BACTERIA FOR CONTROL OF PHENOL SPILL (JANUARY 1975)

			Disappearance rates (d^{-1})			Environmental Conditions[2]		
Aquarium number	Conditions	Phenol/ VSS mass ratio (mg/mg)	Obsd. decay rate	Dilution rate	Biol. Decomp. rate	Avg. pH	Avg. D.O. (mg/l)	Avg. temp. (°C)
1	Phenol	-	0.576	0.528	0.048	7.4	8.8 (8.5)[2]	20.5
2	Phenol + bacteria	1.9	0.984	0.446	0.538	7.5	7.2 (4.5)[2]	18.6
3	Phenol + bacteria + nutrients	1.9	1.656	0.514	1.142	7.6	5.6 (0.3)[2]	20.3

[1] Phenol added = 21,440

[2] Average over a 30-day period.

[3] Lowest D.O. value

Methanol --

Following the aquaria tests with phenol, the methanol tests could be designed with some confidence to provide information on decay rates and effects. Tests were conducted to determine the influence of methanol/VSS mass ratios and the influence of nutrients on removal rates.

Effects of methanol/VSS mass ratio test No. 1--In June 1974, the first methanol test in the aquaria was carried out. It was designed to examine the effects of the methanol/VSS mass ratio on methanol removal rates with aeration. Six aquaria were involved in the test; the initial conditions and results are summarized in Table 24.

Initial concentrations of methanol ranged from 1,000 to 10,000 ppm and the mass ratios ranged from 38 to 1934 mg/mg. While the methanol removal rates ranged from 0.02 to 0.48 d^{-1}, there appears to be little correlation between the mass ratios and the removal rates. As with phenol removal, low dissolved oxygen conditions may inhibit the bacteria and lower the removal rates. Such could have been the case for methanol removal in Aquaria 7, 8, and 9. It is interesting to note that methanol removal in Aquarium 9, to which no bacteria were added, was very high; apparently, bacteria in the aquarium were readily able to break down the methanol.

TABLE 24. EFFECTS OF METHANOL/VSS MASS RATIO ON METHANOL REMOVAL RATES. TEST NO. 1 (JUNE 1974)

			Disappearance rates (d^{-1})			Environmental conditions[1]		
Aquarium number	Initial methanol conc. (ppm)	Methanol VSS mass ratio (mg/mg)	Obsvd. decay rate	Dilu. rate	Biol. decomp. rate	pH	D. O. (mg/l)	Temp. (°C)
1	5,000	193	0.653	0.465	0.188	8.1	8.8	21.3
2	1,000	38	0.684	0.423	0.261	8.3	8.8	20.9
3	10,000	379	0.635	0.459	0.176	8.4	8.8	21.5
7	5,000	1,934	0.898	0.408	0.490	8.9	7.2 (1.0)[2]	21.4
8	1,000	379	0.510	0.493	0.017	9.2	8.0 (2.0)[2]	21.3
9	5,000	-	0.904	0.446	0.458	9.0	6.5 (1.2)[2]	21.5

[1] Average over a 30-day period.

[2] Lowest D.O. value.

Effects of methanol/VSS mass ratio test No. 2 -- The second test to examine the effects of the methanol/VSS mass ratio was conducted in February 1975. Three different mass ratios ranging from 1.75 to 349 mg/mg were used with initial methanol concentrations ranging from 5,000 to 100,000 ppm. Aquarium 1 received only methanol, while Aquaria 4, 5, and 6 each received equal amounts of bacteria and different amounts of methanol. To avoid inorganic nutrient limitations, $NaHCO_3$, KH_2PO_4, and $(NH_4)_2SO_4$ were each added twice a day to Aquaria 4, 5, and 6.

The results of the test (Table 25) indicate some biological removal in each aquarium, with the lowest rate in Aquarium 1 as expected. The removal rates in Aquaria 4 and 5 were relatively low, but apparently the dissolved oxygen concentration, which dropped to near zero in both aquaria within 24 hours after the spill, inhibited removal. Somewhat surprising was the removal of methanol at the initial concentration of 100,000 ppm. Biological activity was present as the dissolved oxygen dropped to near zero in Aquarium 6 after 72 hours, but removal was apparent, perhaps through air stripping, immediately after the spill of methanol.

It is possible that some removal was a result of aeration and, as shown in Section 8, this could account for a substantial portion, $0.23 d^{-1}$, of the observed rates in Aquaria 1 and 5. However, the aeration rate was not of the magnitude used in the experiments described in Section 8. Thus, it is assumed that loss through aeration would be less than $0.23 d^{-1}$ and perhaps negligible.

TABLE 25. EFFECTS OF METHANOL/VSS MASS RATIO ON METHANOL REMOVAL NO. 2 (FEBRUARY 1975)

			Disappearance rates (d^{-1})			Environmental conditions[1]		
Aquarium number	Initial methanol conc. (ppm)	Methanol VSS mass ratio (mg/mg)	Obsvd. decay rate	Dilu. rate	Biol. decomp. rate	pH	D. O. (mg/l)	Temp. (°C)
1	5,000	-	0.70	0.53	0.17	9.4	9.0	19.1
4	5,000	1.75	1.14	0.52	0.62	8.7	1.0	19.1
5	10,000	34.9	0.77	0.48	0.29	7.3	1.0	19.8
6	100,000	349.4	0.96	0.50	0.46	7.7	4.6	20.3

[1] Average over a 30-day period.

Effects of nutrient additions -- The effects of nutrient additions following a spill of methanol were studied in a test carried out in January 1975. Using initial methanol concentrations of 5,000 ppm and a methanol/VSS mass ratio of 26.6 mg/mg, Aquarium 1 received only methanol, Aquarium 7 received methanol plus acclimated bacteria, Aquarium 8 received methanol plus bacteria plus nutrients, and Aquarium 9 received methanol plus bacteria plus twice the mass of nutrients placed in Aquarium 8. The nutrients were added twice each day to Aquarium 8 and 9 to produce the indicated concentration upon each addition. The aquaria were also aerated continuously.

From the results given in Table 26, it is apparent that nutrient additions aided biological removal since the biological removal rate in Aquarium 8 (with nutrients) was twice that in Aquarium 7 (without nutrients). However, doubling the nutrient concentrations did not produce an increase in the decay rate, because either: (1) nutrient concentrations were at saturation levels for growth or (2) low dissolved oxygen levels, (even with aeration) were inhibitory.

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TABLE 26. EFFECTS OF NUTRIENT ADDITIONS ON METHANOL REMOVAL (JANUARY 1975)

				Disappearance rates (d^{-1})			Environmental conditions[1]		
Aquarium number	Conditions	Initial methanol conc. (ppm)	MeOH/VSS mass ratio (mg/mg)	Obsvd. decay rate	Dil. rate	Biol. decomp. rate	pH	D.O. (mg/l)	Temp. (°C)
1	Methanol	5,000	26.6	0.64	0.48	0.16	8.2	8.9 (8.4)[2]	21.6
7	Methanol+ bacteria	5,000	26.6	0.72	0.52	0.20	7.3	6.3 (3.4)[2]	21.6
8	Methanol+ bacteria+ nutrients	5,000	26.6	1.03	0.53	0.50	7.2	3.0 (<1.0)[2]	21.8
9	Methanol+ bacteria+ 2x nutrients	5,000	26.6	0.94	0.52	0.42	7.3	1.8 (<1.0)[2]	22.2

[1] Average over a 30-day period.

[2] Lowest D. O. value.

POND TESTS

RATIONALE

Following the aquaria tests from which initial experience had been gathered on the feasibility of the biological countermeasure, pond tests were conducted to provide additional experience in systems with a larger surface to volume ratio than the aquaria. This experience was desired prior to the tests to be conducted in a model lake.

The nature of the pond spill tests was to be the same as for the aquaria tests. The primary difference was that no water flowed through the ponds. Thus, the equation used for calculating decomposition rates was Equation 74 without the dilution rate term, or:

$$S=S_0e^{-kt},$$

where: $S_O = \frac{M}{V}(M/L^3)$

V = pond volume (L^3),
M = mass spilled (M),
k = decomposition rate (l/T), and
t = time (T).

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Procedures

The ponds were rigid, plastic wading pools arranged in two rows and equipped with banks of fluorescent light to provide 300 to 400 foot-candles at the water surface. They were filled to a depth of about 10 cm with sediment and to a depth of about 12 cm with water and were planted with Vallisneria, a rooted aquatic plant. The surface area of each pond was 2.0 m^2 (diameter of 1.6 m) and the average volume was 312 l. Mixing in each pond was achieved by submersible pumps, resulting in circular water movement. Vigorous aeration was also used in several ponds.

The general experimental procedure was to spill methanol or phenol, followed immediately by the bacteria culture into a pond. The bacterial culture was acclimated to phenol over a one-week period in a batch reactor then transferred to a 200-liter container for further growth. After stirring to ensure initial mixing, the concentration of the spilled chemical was monitored in the pond by TOC, TOD, and gas chromatography analyses. TSS, VSS, pH, temperature, and dissolved oxygen were also monitored. A variation of this pattern was the use of a portable treatment unit to which pond water was pumped, treated by an acclimated culture, and returned to the pond by gravity flow. In this case the spilled chemical concentration was also monitored in the treatment unit effluent.

At the end of each test, the chemical concentration data were plotted and the removal rate calculated using Equation 75. Averages of pH, temperature, and dissolved oxygen were computed.

Results

Methanol Spill Tests —

A simulated methanol spill was carried out in June 1974 in Ponds 1, 2, 3, and 5. Two concentrations of methanol (1,000 and 5,000 ppm) and four bacterial culture concentrations (0, 1.0, 3.0, and 5.0 liters of culture of 665 mg/l VSS) were used. The initial conditions of the test are given in Table 27.

The study was conducted over an eleven-day period, and the results are given in Table 27. Because of the relative equality of the decay rates, it is difficult to determine if biological decay, volatilization, or sorption processes were important. There were increases in VSS concentrations on the third day in Ponds 1 and 2 and on the fifth day in Ponds 3 and 5, indicating bacterial growth. Also, decreases in dissolved oxygen in Pond 5 after day 5 indicated continued growth. The decay rates were substantially less than those measured in the aquaria, but initial VSS concentrations were apparently too small to provide an inoculum larger than that already existing in the ponds.

That minimum inocula are required for methanol removal in aquatic systems was shown in this test.

TABLE 27. RESULTS OF METHANOL SPILL INTO PONDS (JUNE 1974)

Pond number	Initial Methanol conc. (ppm)	Methanol added (kg)	Initial culture conc. (mg/l)	Bacteria culture added (mg)	MeOH/VSS ratio (mg/mg)	Decay rate (d^{-1})	Environmental Conditions pH	D.O. (mg/l)	Temp. (^oC)
1	1,000	0.29	0	0	-	0.15	8.1	8.0(5.9)[2]	25.2
2	1,000	0.29	5.4	1995	145	0.13	8.2	7.0(6.0)[2]	25.7
3	5,000	1.45	1.8	665	2180	0.11	8.2	6.8(5.4)[2]	26.0
5	5,000	1.45	9.0	3325	436	0.10	8.0	4.0(0.2)[2]	26.3

[1] Average over a 30-day period.

[2] Lowest DO value.

Phenol Spill Tests --

The overall purpose of the phenol spill experiment conducted in November 1974 was to test the biological countermeasure on phenol in a larger container than the aquaria. Secondary objectives were to examine the effects of using acclimated as opposed to unacclimated bacteria and to evaluate use of a portable treatment unit containing acclimated bacteria.

The ponds used, the treatment technique applied, and the initial phenol and bacteria concentrations are given in Table 28. Pond 6 was used as a control, receiving phenol but no bacteria, while Pond 7 received phenol and acclimated bacteria. Ponds 8 and 9 received two levels of unacclimated bacteria acquired just prior to the test from the Govalle Treatment Plant. Initial phenol concentrations ranging from 148 to 170 ppm and initial bacteria concentrations from 128 to 224 mg VSS/l were used. Phenol to bacteria VSS ratios ranged from 0.71 to 1.4 mg/mg.

The observed decay rates are given in Table 28 and show that essentially no removal occurred in the control pond (0.02 d^{-1}), while rapid removal took place in the pond with acclimated bacteria (0.76 d^{-1}), even though the dissolved oxygen levels dropped to zero for a short period. The two ponds receiving the two levels of unacclimated bacteria had low removal rates (0.08 and 0.15 d^{-1}, respectively), but the rates were in proportion to the amount of bacteria added. The removal rate in Pond 3, treated by the portable treatment unit, was 0.10 d^{-1}. Since this removal rate was in part a function of the pumping rate of pond water through the treatment system, one cannot directly compare this rate with those from the other ponds. Removal in the treatment unit itself was as high as 90 percent initially, but decreased to about 20% after 10 days when the influent concentration decreased substantially.

In summary, this experiment confirmed the feasibility of the countermeasure with acclimated sludge, the possible use of unacclimated sludge, and the use of a portable treatment unit under proper conditions. The use of the latter unit is discussed in more detail in Section 11.

TABLE 28. PHENOL SPILL INTO PONDS
(NOVEMBER-DECEMBER 1974)

Pond number	Conditions	Initial phenol conc. (ppm)	Initial bacteria conc. (mg/l)	Phenol/ VSS mass ratio (mg/mg)	Phenol decay rate (d^{-1})	Environmental Cond. pH	DO (mg/l)	Temp. (°C)
3	Port.trtmt.unit	165	0	-	0.10	7.4	5.0(0.5)[2]	17.9
6	Control	166	0	-	0.02	8.0	9.5(8.8)[2]	16.0
7	Accl. culture	153	137	1.2	0.76	7.1	6.0(0.0)[2]	15.6
8	Unaccl. culture	170	128	1.4	0.08	7.8	8.5(7.5)[2]	16.0
9	Unaccl. culture	148	224	0.71	0.15	7.7	8.8(7.0)[2]	16.6

[1] Calculated.

[2] Lowest D.O. value.

MODEL LAKE TESTS

In order to assess, on a larger scale, the candidate techniques for hazardous material spill cleanup developed in laboratory tests, model lake tests were performed. These tests included dye tests to determine general mixing patterns of the model lake and simulated phenol spills with or without barriers, but with bacteria. The tests performed are summarized in Table 29, and are described below.

TABLE 29. MODEL LAKE SPILL TESTS (1976)

TEST	Spill material Dye	Phenol	Sludge Acclimated	Unacclimated	Barrier with	without
Spill I (June 16-17)	x	-	-	-	-	-
Spill II (June 22-24)	x	x	x	-	-	x
Spill III (July 6-9)	x	x	x	-	x	-
Spill IV (July 20-Aug.2)	x	x	-	x	x	-

Dye Spills

Rationale --

As a prelude to a phenol spill, dye was spilled in the model lake to determine the spreading action or dispersion rate and to determine whether stratifications existed. The bottom slope and the circular shape of the model lake perimeter, as well as wind action on the surface of the water, could influence dispersion, while the density of the substance spilled could cause stratification.

Before phenol could be spilled, the best method for simulation of a spill needed to be determined. A dye that could be detected easily and that could be used in small enough concentrations so that it would not interfere analytically with other parameters was needed. The volume of dye necessary for detection had to be determined and a technique had to be found for dispensing the dye.

To evaluate data from future phenol spills, a method for calculating mass balance was developed using the dye tests. This permitted the recovery of the dye and the spilled material mass to be calculated, which, in turn, enabled calculation of the decomposition rate of spilled phenol.

Procedures --

Apparatus — The pond (Figure 42) used for the model lake is a 30.5-m diameter, brick-lined, circular structure that was formerly a clarifier for a magnesium plant. Along the outer perimeter, it is about 2.1 m deep, sloping to a depth of about 3.6 m near the center. Immediately around the center pole is a trench, 0.9 to 1.2 m wide and 1 m deep, making an approximate depth at the pose of 4.6 m. The model lake had a volume of about 1.78×10^6 liters.

A sampling grid (Figure 43) was formed using 0.64-cm (1/4-inch) sash cord stretched across the pond, forming a 9.1-m x 9.1-m grid. Three cords were stretched 3 m apart across the pond in a north-south direction. Three more cords were stretched in an east-west direction. Sample stations were marked with tape at the intersection of the cords.

To sample the various stations, a 3.6-m, flat-bottomed, aluminum boat was used. It was propelled by pulling along the sash cord grid. Water samples were taken with a 1.0-liter Van Dorn sampler at various depths. To determine the temperature and dissolved oxygen, a Yellow Spring Instrument Oxygen meter, Model 51A, was used.

A Turner Fluorometer was used to detect the concentration of dye present in the water samples.

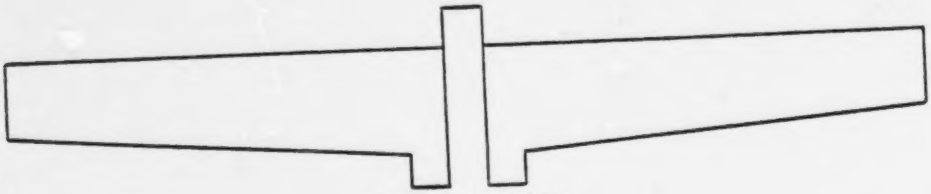
Methods --

Spill--In order to introduce the dye into the water column, a toy balloon was filled with 300 ml of liquid Rhodamine B dye. Rhodamine B dye was used because of the low concentrations (ppb) that could be detected on the fluorometer.

The dye-filled balloon was tied to a rope and lowered to a depth of 1.2 m at station No. 22. The balloon was then burst at 0800 hours on the day of the test with a broken piece of glass fastened on the end of a pole.

Sampling--Water sampling was begun one hour (0900) after dispensing the dye and additional samples were taken at 1200, 1600, and 2200 on the day of the spill and at 0800 on the following day.

The water samples were taken from a boat at 3 depths-0.15 m and 1.2 m below the surface and at 0.3 m off the bottom. The stations sampled at these depths were 11,



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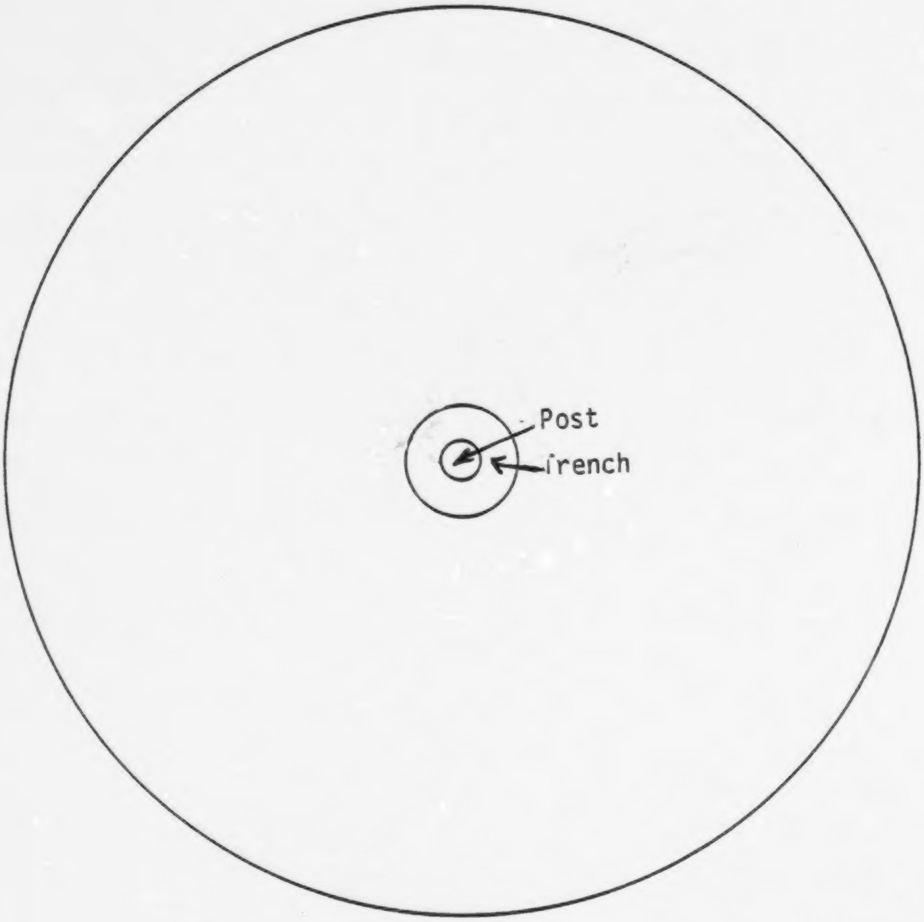
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Top view

Figure 42. Schematic diagram of model lake.

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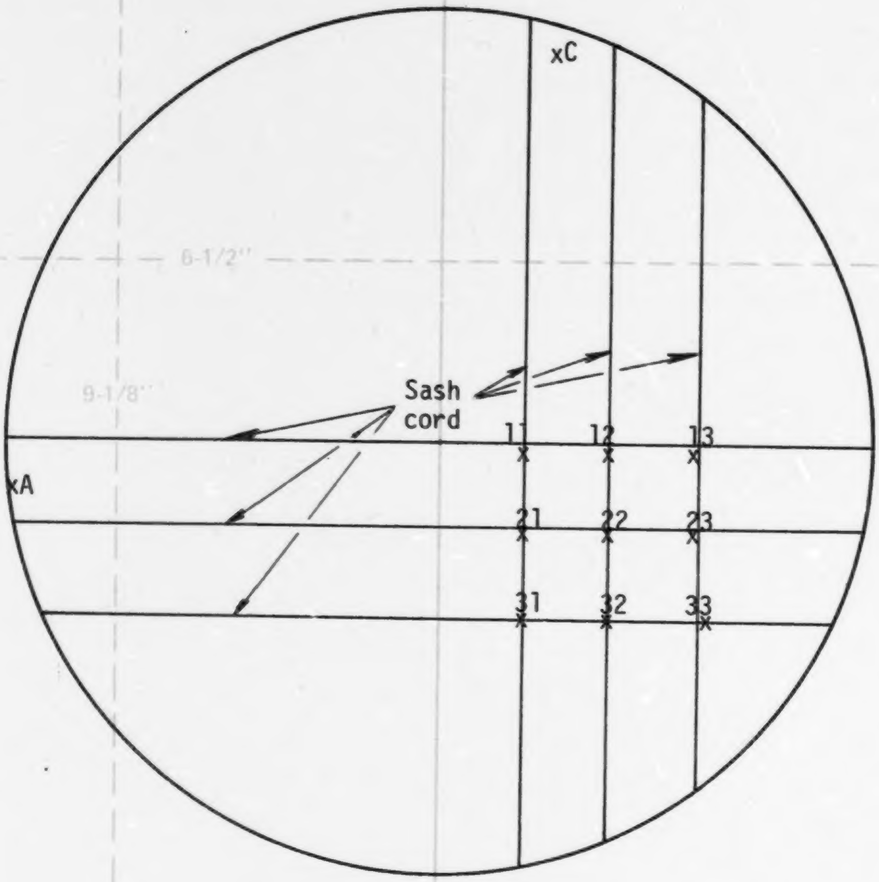
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Figure 43. Diagram of sampling stations

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12, 13, 21, 22, 23, 31, 32, and 33. Stations A and C were each sampled from the shore at 0.15 m and 1.2 m below the surface.

The dissolved oxygen and temperature were determined at all three depths only at station 22.

Weather observations were made at each sampling time. Approximate wind velocity and direction and cloud cover were noted.

Plastic bottles (125 ml) were filled from the Van Dorn sampler. A total of 31 samples was taken at each sampling time, one representing each depth and station described previously. The excess water from the Van Dorn sampler was emptied into a large plastic can for disposal on shore away from the pond.

Analysis of samples-- Before the sample could be analyzed on the fluorometer, standard curves had to be constructed relating meter fluorescence units and dye concentration. To do this, the following volume to volume concentrations of dye were made: 25, 50, 100, 200, 300, 400, 500, 600, 800, 1,000, 1,200, and 1,500 (ul/l). These were read on the fluorometer at the proper range, and a curve was plotted for each range as shown in Figure 44. These curves were then used for determining the concentration of dye present in each water sample.

The samples that had been collected were stored in the dark and were temperature stabilized. At completion of the sampling, the fluorescence of the samples was measured with the Turner Fluorometer. The concentrations (ppb) of the samples were determined from the previously prepared standard curves.

Data analysis--Concentrations of the samples from each station and depth were calculated. Cross-sectional drawings were made in the east-west vertical plane, the north-south vertical plane, and the horizontal plane. On these drawings were recorded the concentration at all stations and depths. There was a set of drawings for each sample time. Contour lines were then drawn to indicate the various concentration patterns.

To determine the percent recovery of spilled dye, the average concentration of the dye in the pond at the last sample period was calculated. Next, the mass of spilled dye was calculated, and finally, using the volume of the pond and the average final dye concentration, the mass of recovered dye was caluclated.

Results--

Dispersion of the dye--It was possible to visually observe initial dispersion of the dye. At approximately two hours after the spill, the dye appeared to cover the entire pond. Based on the data plots, mixing in the pond was complete within 24 hours, except for a slightly higher concentrated area of dye in the deepest part of the pond (Figure 45).

Recovery of the dye-- Some 300 ml of dye were initally spilled into the pond. The average concentration of the dye at the final sampling period was found to be 195 ppb (or 0.2 µl/l). The mass remaining was 356 ml (mass (ul)= V(l) x ul/l = 1.78 x 10^6 lx 0.2 ul/l x 10^3 ul/ ul). It can be concluded that little, if any, of the dye was lost during the spill. Therefore, the use of Rhodamine B dye appeared to be a satisfactory method of establishing a dilution baseline and of "tagging" the phenol during spills.

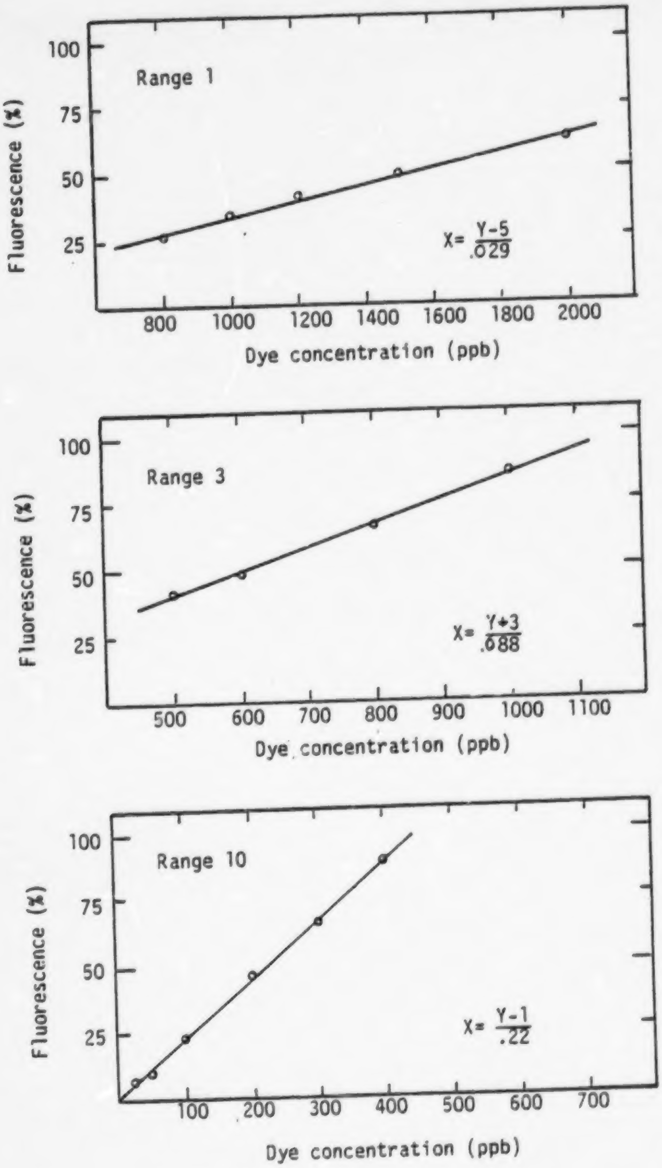

Figure 44. Standard curves for dye concentration

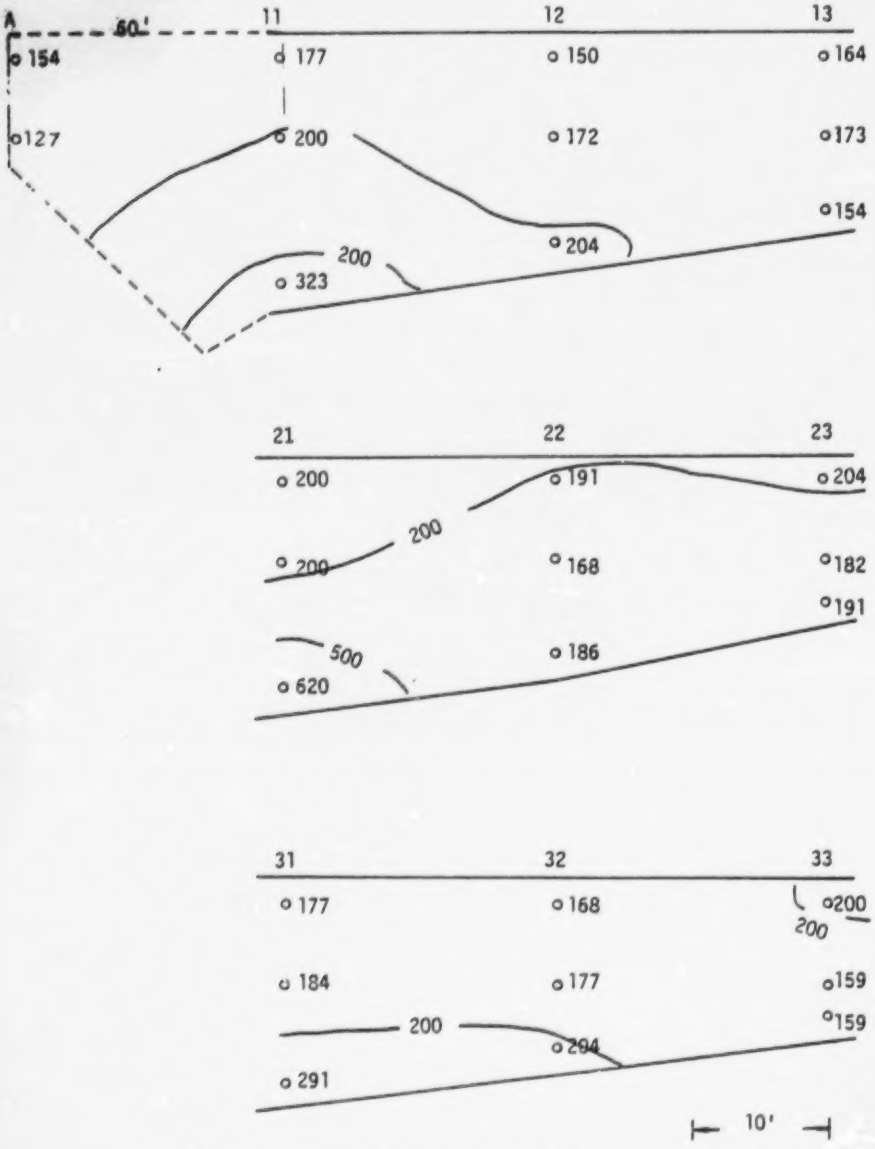
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Figure 45. Pattern of dye dispersion

Phenol/Dye Spill with Sludge and Without Barrier

Rationale--

In the combination phenol/dye test, dye was used to "tag" phenol (estimate dispersion) and to establish a dilution baseline. Visual and analytical tracing of dye dispersion indicated the location of phenol. The establishment of a dilution baseline provided a rate with which to compare the degradation of phenol. It was assumed that phenol would be diluted at a similar rate as the dye.

Acclimated sludge was used in the test, but without a barrier. The purpose of the test was to simulate a natural spill situation in which no barrier was available or in which use of such was not feasible. It was assumed that the spreading action of the phenol/dye and of the bacteria would progress at the same rate.

Observations were made of the effect of the spill on the aquatic organisms in the pond. The acute toxic effect on the fish could be readily observed.

Procedures--

Apparatus-- Following the previous dye test, the pond was pumped out to a depth of approximately 0.3 m and then refilled to the original level with groundwater. The same grid as before was used, but an additional sash cord was added in the east-west direction 9.1 m south of the other ropes (Figure 46). A larger sampling area was made.

The other apparatus — boat, temperature and dissolved oxygen meter, and fluorometer -- were the same as in the previous test. To detect phenol (76% of which is carbon), the Beckman 915 Total Organic Carbon Analyzer was used. With this instrument, total carbon, inorganic carbon, and total organic carbon present in the samples were determined.

To determine the total and volatile suspended solids in each sample, a Millipore vacuum filtering apparatus with Grade 934AH glass-fiber filter papers was used.

A 104°C drying oven was used to dry the samples and a 600°C muffle furnace was used to drive off the volatile solids.

Methods —

Approximately 190 liters of activated sludge was obtained from the aeration basin at the Govalle Sewage Treatment Plant in Austin, Texas. The sludge was acclimated with aeration in a 208-l drum. During the period of acclimation, nutrients needed to sustain bacterial growth, as shown in Table 30 A and B, were fed to the sludge daily. Phenol was fed in increasing amounts and glucose was fed in decreasing amounts (Table 30 B) during acclimation, furnishing the carbon source necessary for maintaining growth. Immediately before the spill, the total suspended solids and the volatile suspended solids were determined on the acclimated sludge. Also, 17 liters of phenol were mixed with approximately 80 liters of water in a 208-l drum. It was assumed that the phenol would go into solution with this water and thus go into

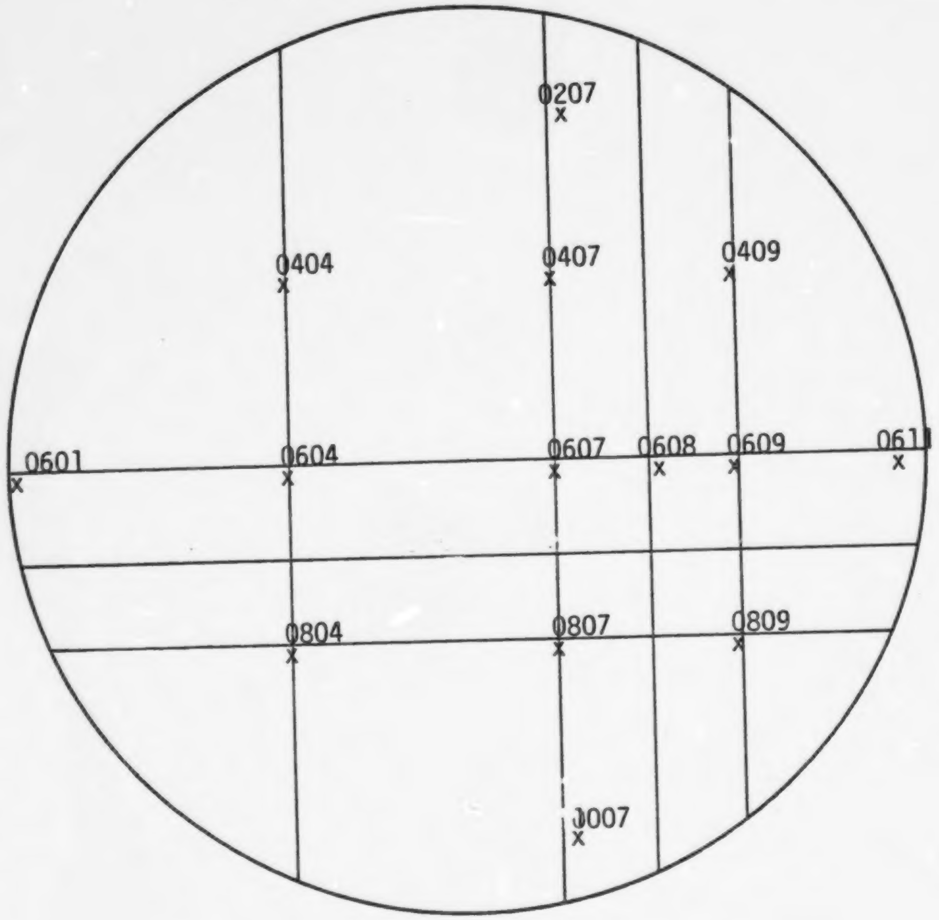
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Figure 46. Diagram of sampling stations, phenol dye spill with sludge and without barrier

solution more readily with the water in the pond. To the water-phenol mixture, 250 ml of Rhodamine B dye were added.

The drum of phenol/dye solution and the drum of acclimated sludge were positioned beside the pond. Using a Little Giant model 3E-12R submersible pump with 15.2 m of garden hose attached, the phenol/dye solution was pumped into the pond at station 0608. The end of the garden hose was barely submerged.

After spilling the phenol, the sludge was pumped in a similar manner, spreading it across the surface in an approximate 3.0-m radius around the boat.

Water sampling was begun one hour (0930) after the spill. Additional samples were taken at 1230, 1630, and 2200 on the day of the spill, at 1000 on the first day after the spill, and 0900 on the second day after the spill.

Samples were taken as before at 0.15 m below the surface and at 0.30 m from the bottom at stations 0809, 0609, 0409, 0207, 0601, 1007, and 0611. Samples were also taken at 0.15 m below the surface, 1.2 m below the surface, and 0.3 m off the bottom at stations 0608, 0407, 0607, 0807, 0804, 0604, and 0404. All parameters were measured and samples were taken at each depth and station.

Weather observations were made at each sampling time as before. The analysis for determining the concentration of dye in each sample was done as in the previous test. Determination of total suspended solids (TSS) and of volatile suspended solids (VSS) was accomplished by filtering 50 ml of sample through pre-weighed, glass-fiber filter papers with a Millipore vacuum filter apparatus. The filter papers were then dried for one hour at 104°C and weighed to determine the TSS (mg/l). The filter paper was then ignited at 600°C for 15 minutes and reweighed to determine the VSS (mg/l).

Filtrate from the VSS and TSS tests was used to determine Total Carbon (TC), Inorganic Carbon (IC), and Total Organic Carbon (TOC). A 20-ul sample was injected into the appropriate furnace port on the Total Organic Carbon Analyzer. From these injections, TC and IC were obtained. The difference between the two was TOC.

In order to detect toxicity effects on the organisms in the pond, visual observations were made at the time of the first appearance of affected fish and at the time when the last appearance of dead fish occurred.

Results --

From cross-sectional plots of the dye data, it can be seen that within six hours, the upper portion of the ponds was uniformly mixed. However, higher levels of dye were found near the bottom, with the deepest area concentrating the dye. Within 24 hours, mixing in the pond was complete, apparently due to a brisk wind. The dye maintained a fairly constant concentration throughout the test.

From the background samples taken before the spill it was determined that there were 137.2 ppb (or 137 ul/l) of dye in the pond. This represents a total dye volume of 244 ml in the pond. Some 250 ml were added with the spill of phenol bringing the total volume to 494 ml.

Calculations of the average concentration of dye in the pond at the last sampling period showed that 861 ml were recovered. This apparent excess of dye at the test end could not be accounted for.

At the end of the first hour, the phenol settled to the bottom below the spill station. By the 24th hour after the spill, the phenol was dispersed completely. Up to this point the mass of phenol as TOC had shown no significant change. However, at 36 hours there was a significant increase. This was perhaps due to decomposition of aquatic organisms killed by the toxicity of the phenol, resulting in the release of organic carbon into the water column. Because of this analytical problem, the results of the test were considered inconclusive. The acute toxic effect of phenol on fish in the pond was evident. Fish began floating by the end of the first hour and affected fish died within the first 24 hours of the test.

TABLE 30 A. SLUDGE FEEDING/ACCLIMATION SCHEDULES: NUTRIENTS FED DAILY TO SLUDGE

Nutrient	Concentration (g/ℓ)	Amount per 190 l
K_2HPO_4	3.2	608
KH_2PO_4	1.6	304
$(NH_4)_2SO_4$	1.5	285
$MgSO_4 \cdot 7H_2O$	0.15	28.5
$FeSO_4 \cdot 7H_2O$	0.005	0.95
$ZnSO_4 \cdot 7H_2O$	0.005	0.95
$MnSO_4 \cdot H_2O$	0.004	0.76
$CaCl_2$	0.02	3.8

TABLE 30 B. SLUDGE FEEDING/ACCLIMATION SCHEDULE

Day	Phenol (ml/190 ℓ)	Glucose (g/190 ℓ)
1	19	178.6
2	76	142.5
3	152	95
4	228	47.5
5	300	--

Phenol/Dye Spill With Acclimated Sludge and Barrier

Rationale --

To determine the feasibility of using a barrier coupled with the biological countermeasure, this test was conducted. First, it was necessary to know whether a barrier would actually contain the spilled material, thus, the design features for a suitable barrier were considered. It was assumed that since the specific gravity of phenol and of the bacteria was greater than water, the barrier needed to extend from the surface to the bottom of the water column. Second, it was necessary to simulate actual spill and countermeasure application conditions, and third, it was necessary to sample in such a way as to detect phenol decomposition, bacteria growth, and leakage under the barrier.

Procedure --

Apparatus--The barrier was made of a polyethylene sheet (6.1 m x 10.7 m), nine styrofoam floats (0.9 m x 0.2 m x 0.08 m), 10.7 m of 9.5-mm steel chain, and duct tape.

The floats were spaced along the 10.7-m edge of the polyethylene sheet and taped in place. The sheet was folded over the floats and taped to itself. The chain was encased in the same manner along the other 10.7-m edge (bottom). The ends of the polyethylene sheet were overlapped and taped with duct tape forming a cylinder approximately 3 m in diameter with floats along the top and a chain (for weight) along the bottom (Figure 47). The remaining apparatus was the same as for the previous spills.

Methods --

Sludge was again obtained from Govalle Sewage Treatment Plant and acclimated as in the previous test. The model lake was pumped down to within 0.3 to 0.6 m of the bottom and refilled with clean groundwater. The barrier was positioned in the pond with the center at station 0608 (Figure 48).

At 0830 hours, 10 ml of Rhodamine B dye was poured inside the barrier, followed by 10 liters of liquid phenol. The water was stirred with a stirring rod during the addition of phenol. The acclimated sludge was pumped in as before, but with stirring as during the phenol addition. The volume of the expanded cylinder was approximately 22.2×10^3 l. However, stirring resulted in the barrier walls collapsing inward, making the volume inside the barrier substantially less. Pond water was pumped into the barrier in an attempt to re-expand the walls, but was only partially successful.

Water samples were taken using the same methods as before at 1000, 1300, and 1700 hours on the day of the spill, at 0830 and 1600 on the second day, at 0830 on the third day, and at 0830 and 1600 on the fourth day at three depths -- 0.15 m, 1.2 m, and 0.15 m from the bottom. The stations sampled were 0607, 0608, and 0609.

The results for dye, phenol, and VSS concentrations were analyzed in the same manner as for the previous phenol/dye spill.

Results --

A large proportion of the dye remained inside the barrier throughout the test and the dispersion pattern of the dye was very similar to that of phenol. Phenol was retained within the barrier with some leakage under the barrier on the deep side. The TOC data indicated a decrease from 90 ppm to 35 ppm (61%) inside the barrier within four days (Figure 49). Since the TOC outside the barrier maintained a constant background level except at the bottom at Station 0607, where leakage under the

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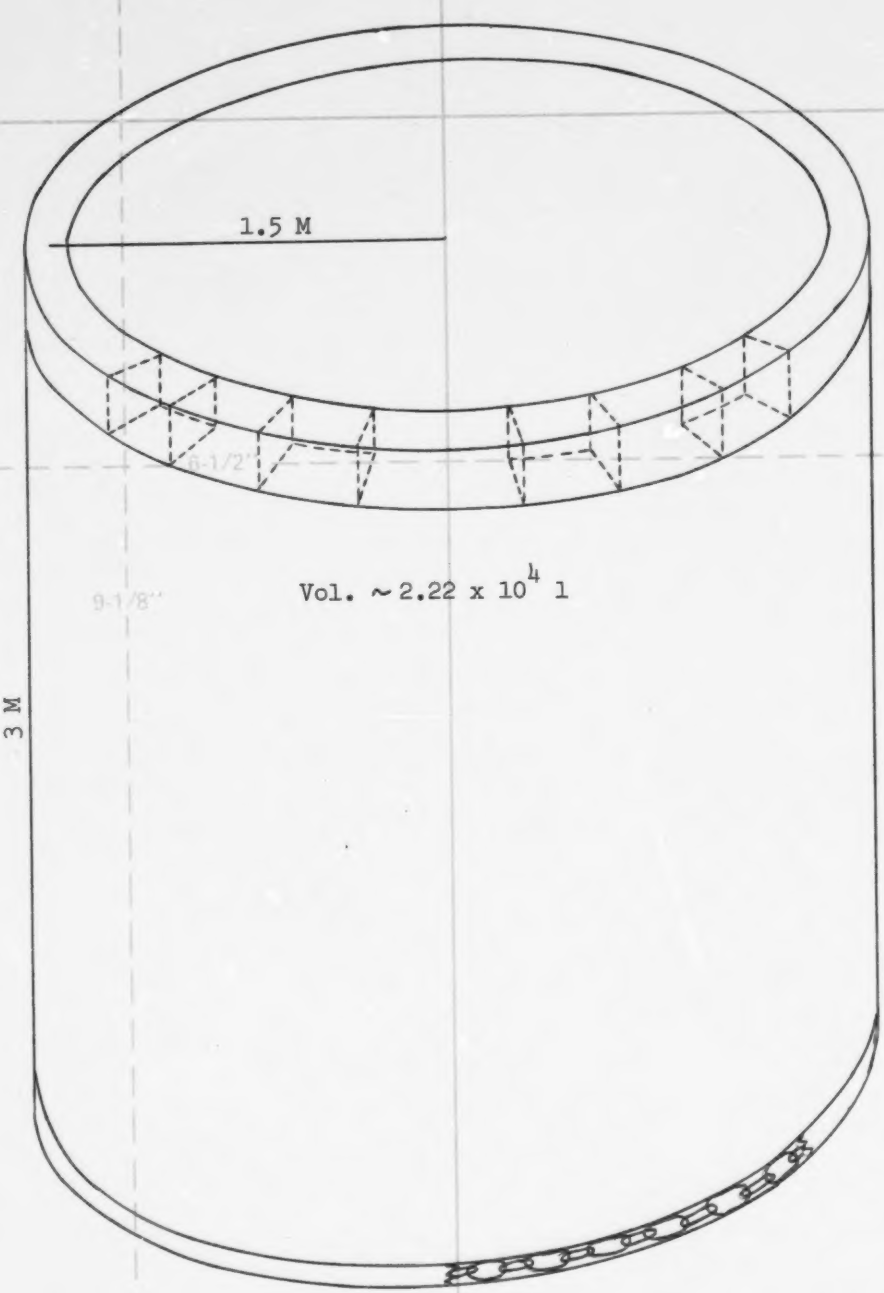

Figure 47. Barrier for phenol/dye spill.

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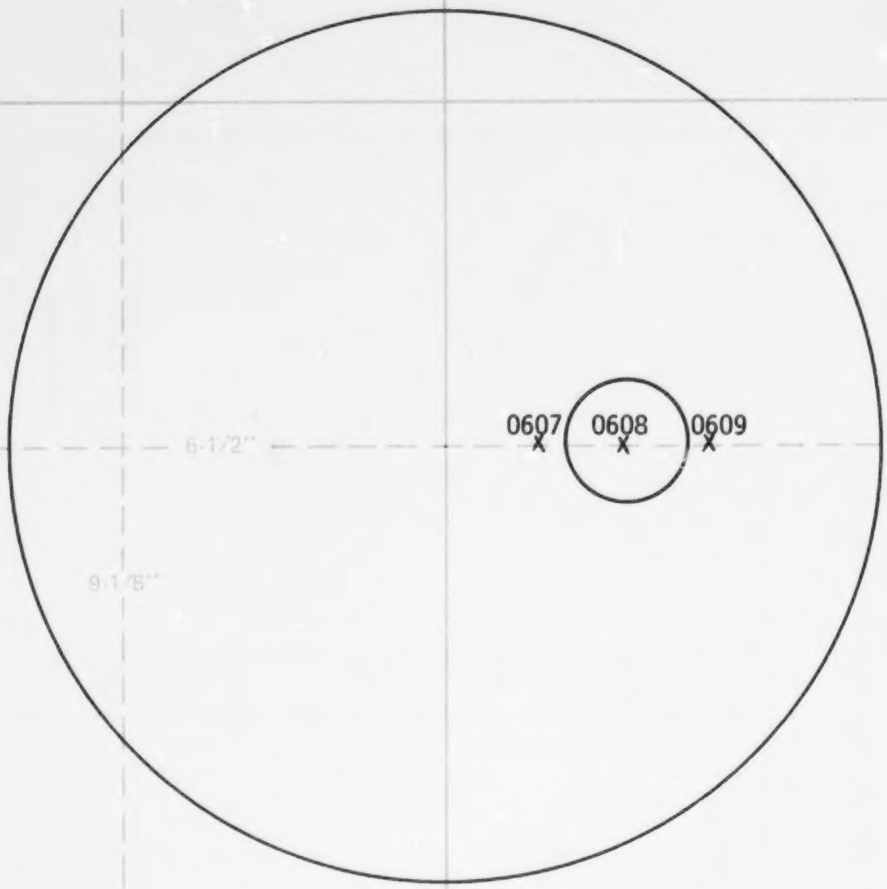
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Figure 48. Sampling stations and barrier position.

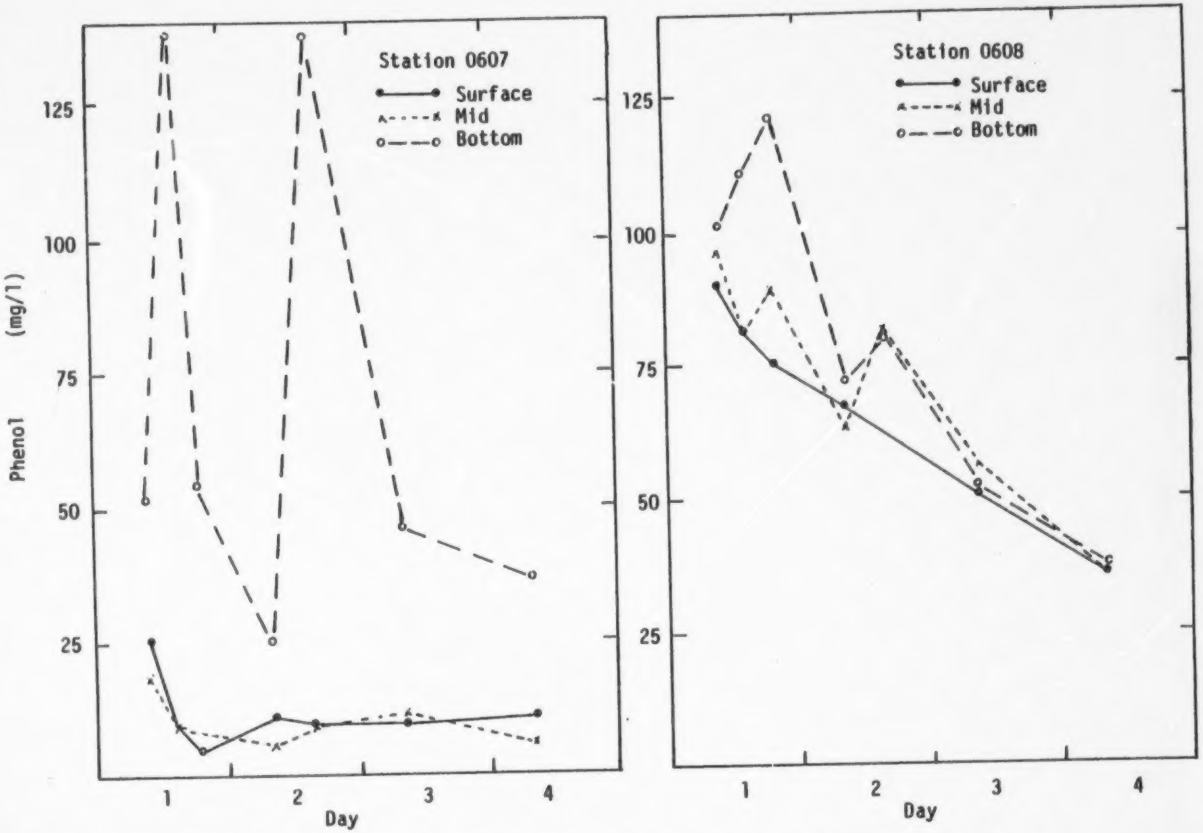

Figure 49. TOC measurements during phenol/dye spill with acclimated sludge.
I. Station 0607, II. Station 0608, III. Station 0609, IV. Surface (cont'd.)

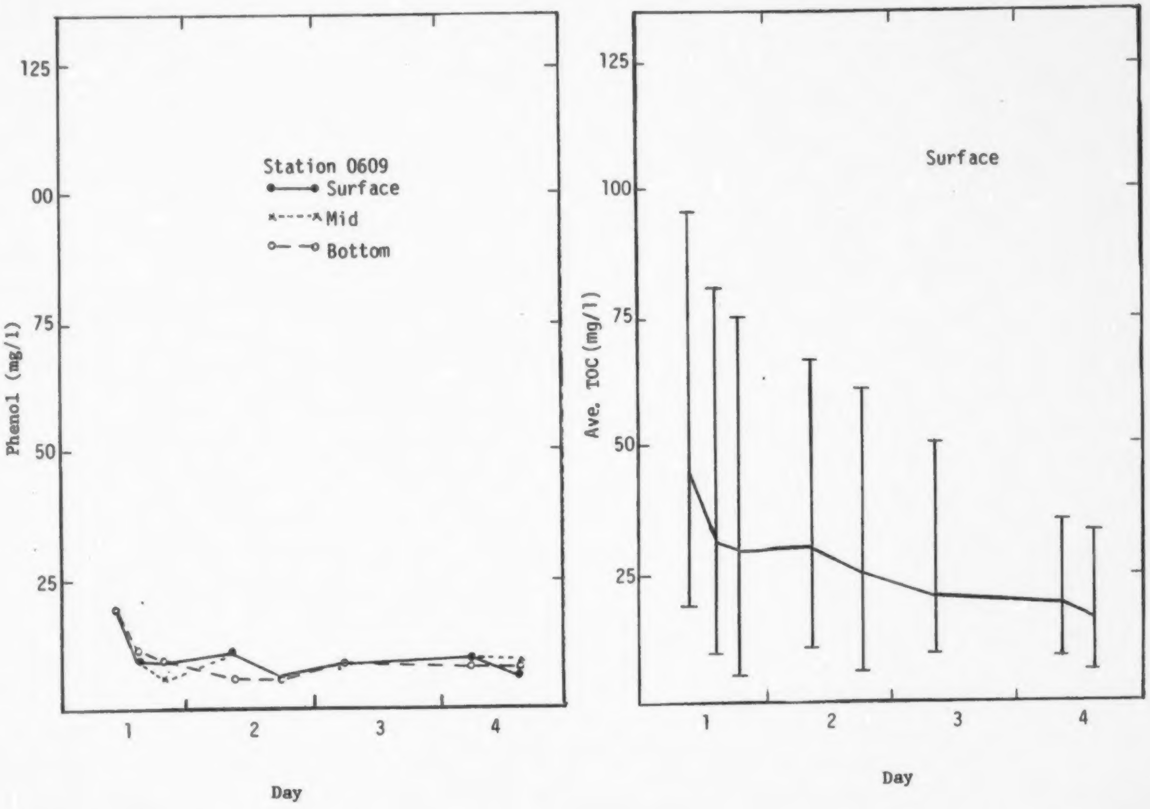

Figure 49. cont'd.

barrier occurred (see Figure 49), it was assumed that the organisms present in the acclimated sludge were in fact decomposing phenol. The rate of decomposition calculated from Figure 47 was 0.24 d^{-1}. A small portion of this rate may be attributed to dilution according to the dye results, although it is possible that the dye was also decomposed or sorbed onto suspended solids.

Twenty-four hours after the spill, the dissolved oxygen at all depths at station 0608 dropped significantly. The dissolved oxygen at mid and bottom depths fell to below 1 mg/l (Figure 50), indicating bacterial utilization of the oxygen. The dissolved oxygen at stations 0607 and 0609 maintained a normal level throughout the test.

Immediately after the spill, TSS and VSS levels in the barrier were high (20-50 mg/l) because of the bacteria added. After 24 hours however, only very small (0 mg/l) concentrations were detected. Over the next 24- to 48- hour period, there was an increase in TSS and VSS concentrations (to 22 mg/l) within the barrier, compared to those levels observed outside the barrier, thereby indicating bacterial growth.

The primary problem encountered in this test was a mechanical one involving the barrier. Stirring action caused the sides to collapse inward. However, even with this problem, the barrier apparently did contain the phenol and bacteria.

Phenol/Dye Spill with Unacclimated Sludge and Barrier

Rationale --

Sludge that had not been acclimated was used in this spill test to determine the feasibility of using readily available sludge directly from the sewage plant, thus reducing the time needed for acclimation. It was assumed that there would be a slight lag in decomposition while in situ acclimation took place, but it was thought that this would still result in rapid decomposition of phenol.

An improved barrier with semi-rigid sides was designed and built. It was assumed that this barrier would maintain its shape but still be maneuverable.

Procedures --

Apparatus —

The polyethylene sheets and floats on the barrier used in the previous test were added to a skeleton constructed of 1.3-cm PVC pipe and T's. Onto each of two 4.9-m by 1.3-cm PVC pipes, were loosely slid two T'S (1.3 cm x 1.9 cm x 1.9 cm). The ends of these two pipes were then connected with 1.3 cm T's, thus forming a circular structure. A second circular structure exactly like the above was made. Six 1.8-m lengths of 1.3-cm PVC pipe were then connected to the T's of each circular structure, becoming the rigs of the cylindrical skeleton (Figure 51). The skeleton was slid inside the polyethylene barrier, positioned with the top skeletal ring just under the floats, and then taped into place. The barrier dimensions were approximately: diameter3.0 m, circumference-9.8 m, and height- 2.4 m. This barrier was then located with its center at station 0608.

The remaining apparatus was the same as for the previous spills.

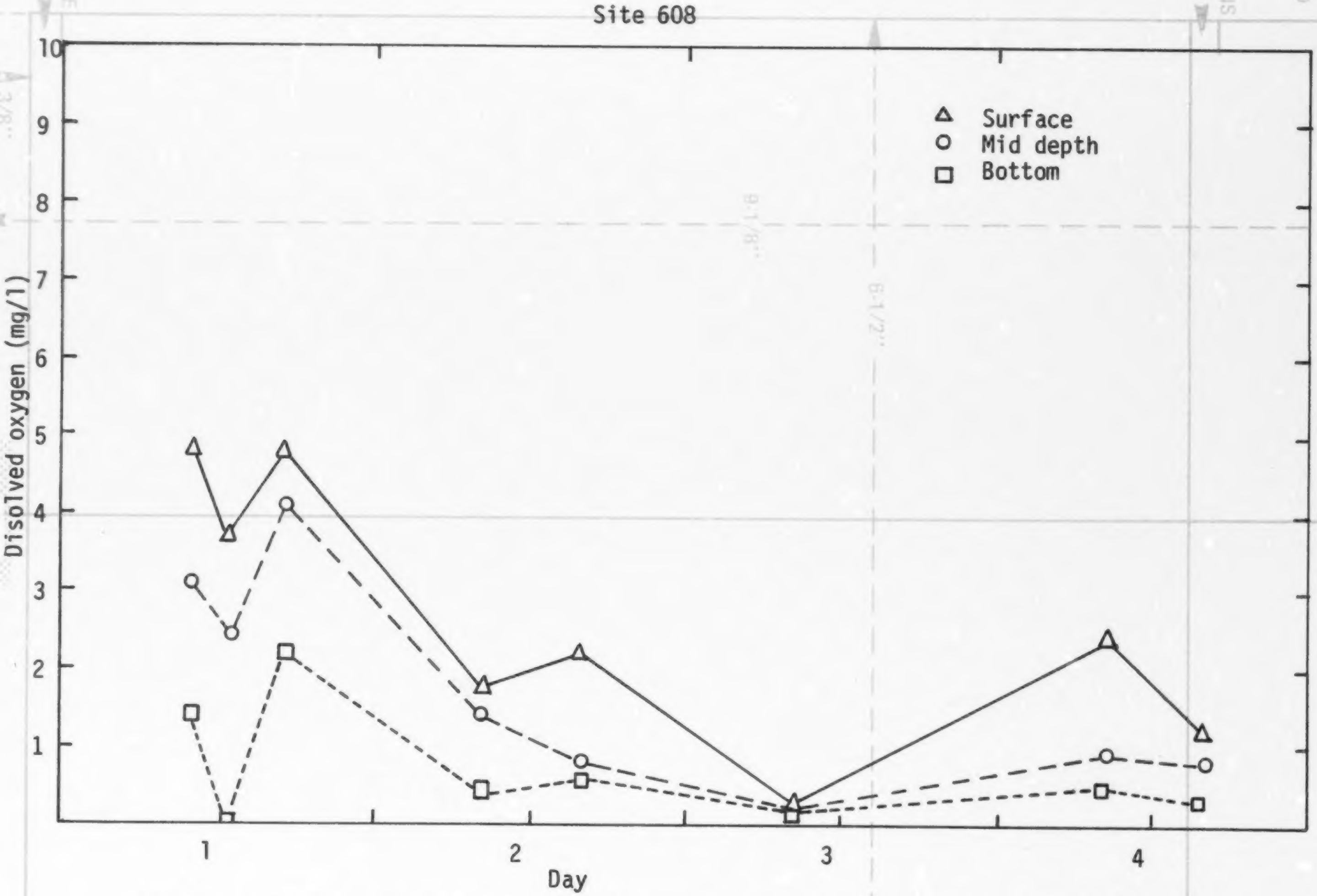

Figure 50. Dissolved oxygen levels following phenol/dye spill with acclimated sludge in model lake.

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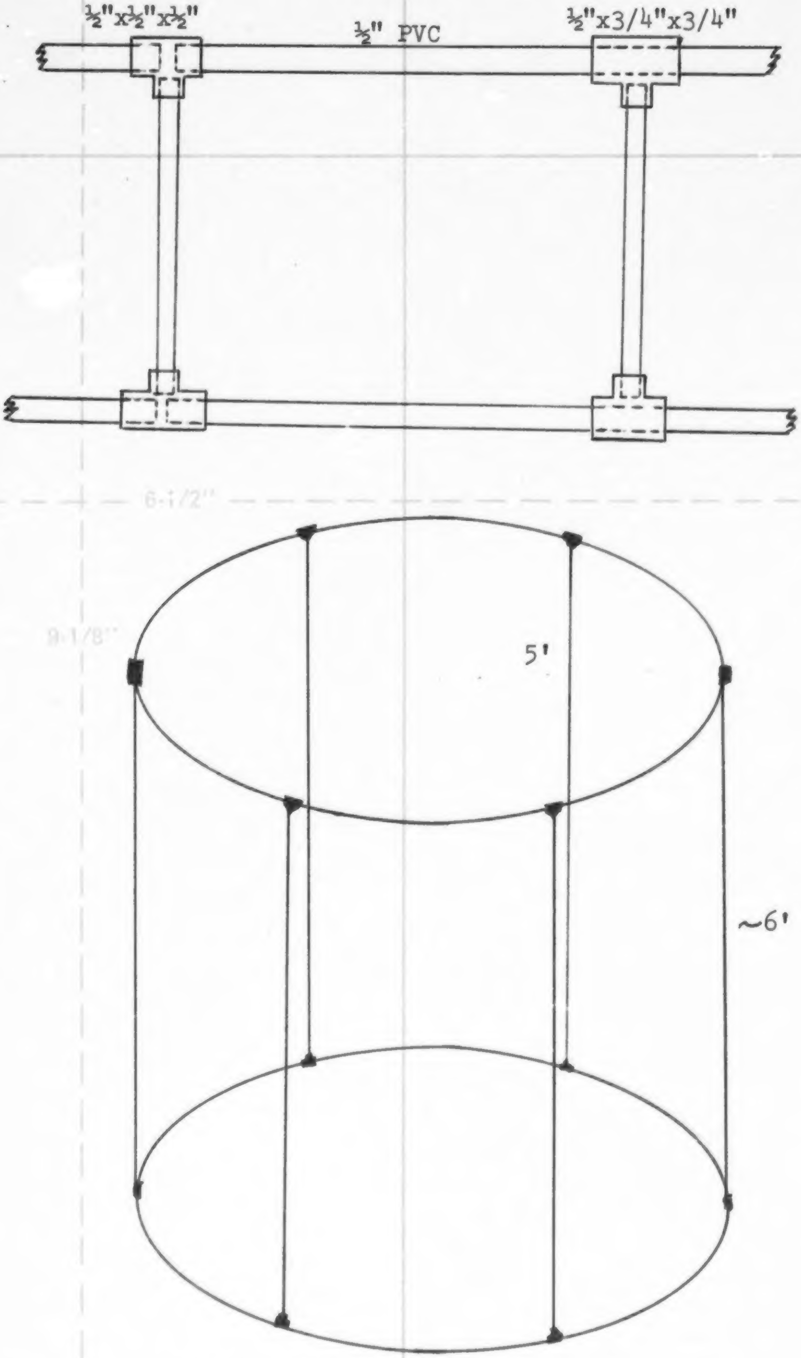

Figure 51. Diagram of barrier with semi-rigid sides.

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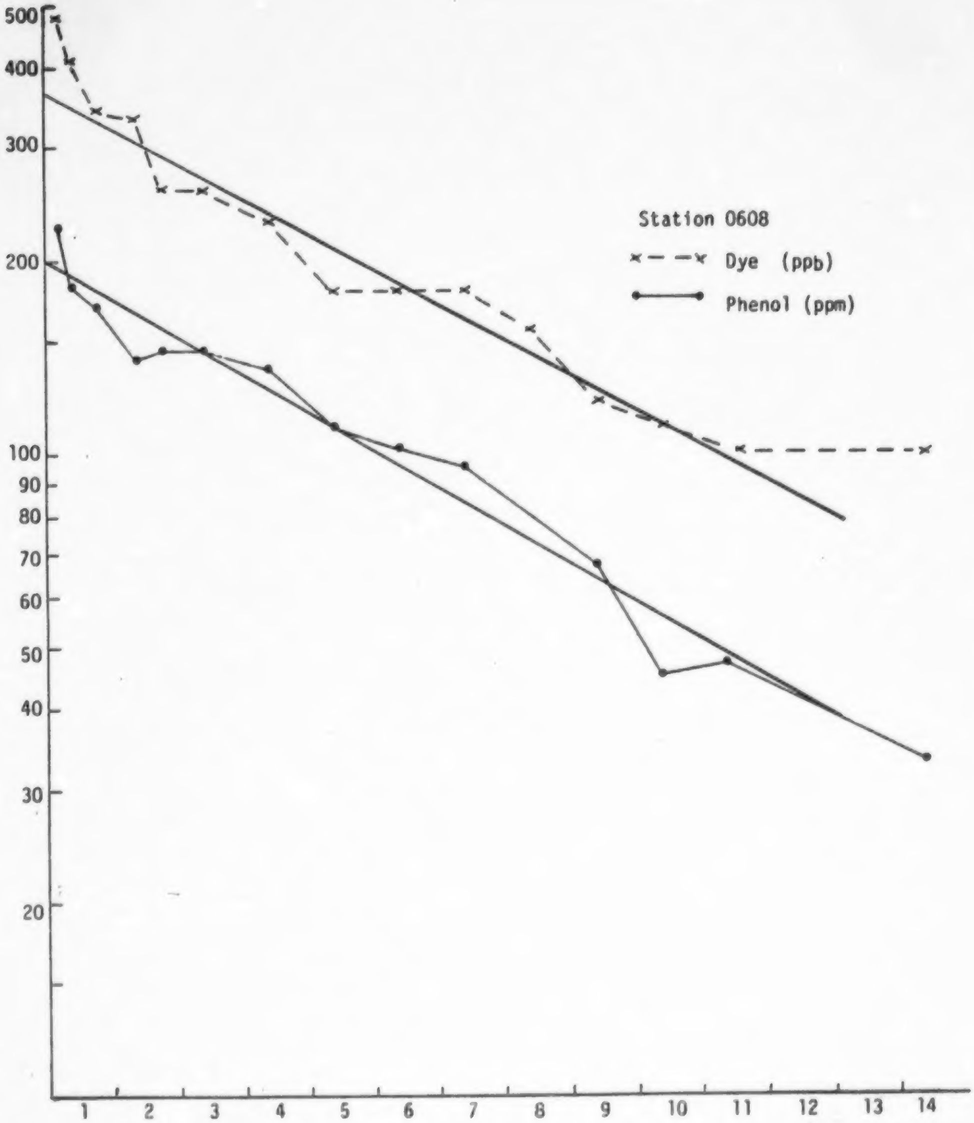

Figure 52. Phenol/dye disappearance in model lake tests with unacclimated sludge.

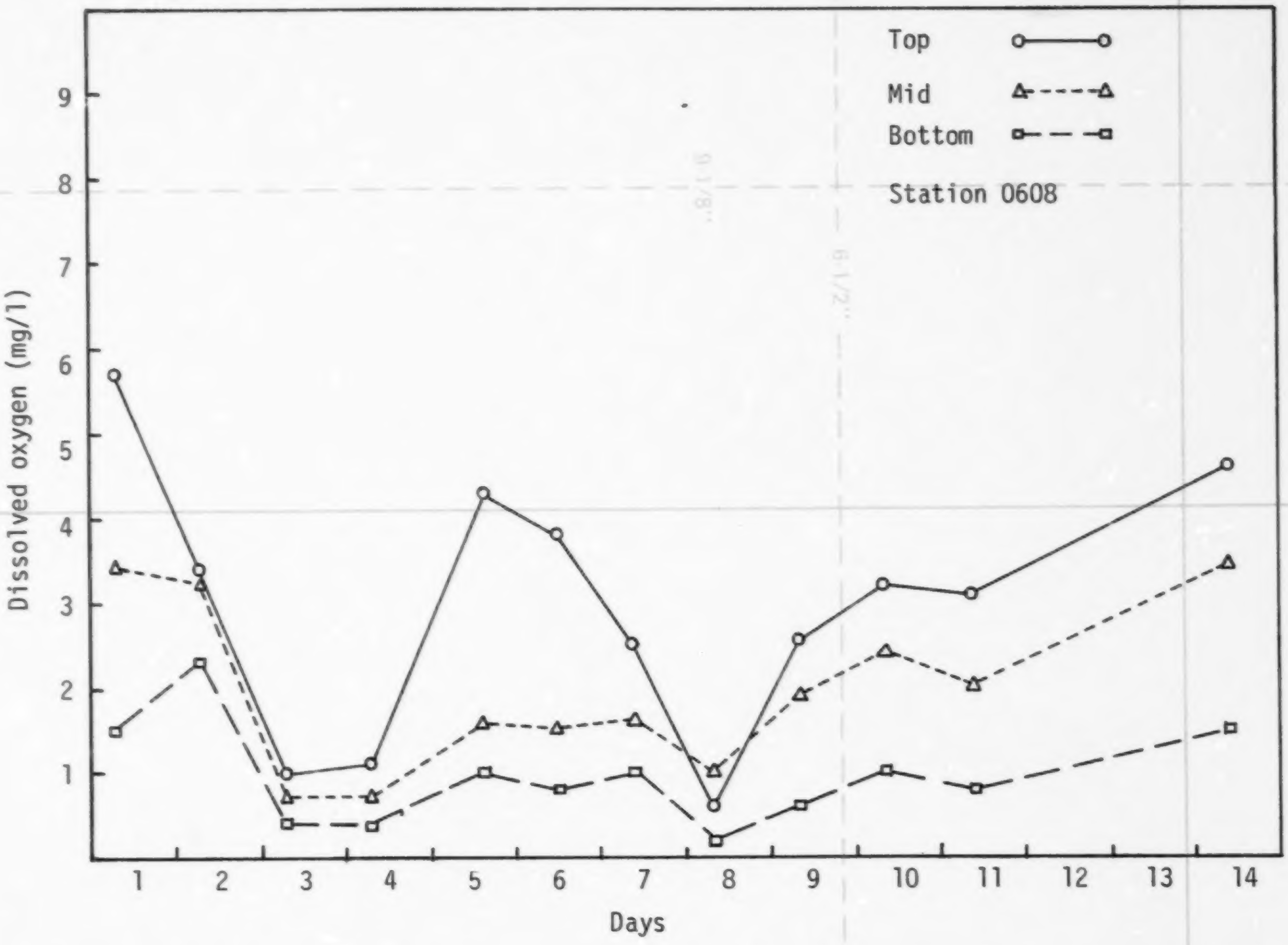

Figure 53. Dissolved oxygen concentrations following phenol/dye spill in model lake with unacclimated sludge.

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Methods —

Since there was no acclimation of bacteria, on the day prior to the spill approximately 190 l (50 gal) of activated sludge were obtained from the aeration basin at the Govalle Sewage Treatment Plant. The sludge was returned to the laboratory and aerated overnight.

The water in the pond was pumped down and refilled with groundwater before the spill and the improved barrier was put into place.

Immediately before the spill, 10 ml of Rhodamine B dye were mixed with 10 liters of liquid phenol and at 0830 hours this mixture was poured into the center of the barrier. The unacclimated sludge was pumped in as before, but there was no stirring during the spill. The sludge was spread about the surface with a hose to insure coverage of the spill area.

Water samples were taken on July 20, 1975 at 0930, 1200, and 1600 hours, on July 21 at 0830 and 1600 hours, and on July 2 through August 2 (omitting July 31 and August 1) once daily at about 0900 hours. Samples were taken at the same depths and stations as in the previous test.

Results —

The dye remained primarily within the barrier as in the previous test and it was assumed that the phenol was also contained. The rate of decrease of phenol (measured as TOC) was substantially less than in the previous test. At the end of the eighth day, the concentration of phenol (TOC) had decreased by about 50% (Figure 52) and by the end of the fourteenth day there was a total decrease of 67%. In contrast, in the previous test using acclimated sludge, it took four days to obtain 50% reduction in the phenol concentration. The rate of phenol reduction was d^{-1} based on the data shown in Figure 51. It was also apparent that the dye decreased at approximately the same rate. Based on the sampling data at stations 0607 and 0609 outside the barrier, no significant amount of phenol or dye was lost under the barrier, thus the decrease in dye concentration cannot be attributed to dilution. With this conclusion about the dye, it was also concluded that the phenol was not diluted, but was decomposed.

By the third day of the spill, the dissolved oxygen at all depths of station 0608 reached a concentration of less than 1 mg/l (Figure 53) and this level was maintained for a period of 48 hours, indicating a high level of bacterial action. With the exception of the eighth and eleventh days, there was a steady increase in DO from the fifth day to the end of the test. The two exceptions could be the result of a response to cloud cover, which would decrease the production of oxygen by algae in the system. The steady increase in DO indicates recovery of the system from the effects of the spill. Stations 0607 and 0608 maintained a high DO concentration as would be expected in a normal system.

The barrier used in this test proved very satisfactory. It maintained its shape well and, as previously discussed, apparently contained the spilled material.

SECTION 10

COUNTERMEASURE STORAGE

One of the deficiencies of the biological countermeasure often mentioned is the problem of storage. It had been assumed (Dawson et al., 1972) that liquid storage would be necessary, but storage in a dry, powdered form is also possible. The experimental work described in this section focused on showing the feasibility of storage methods using freezing and lyophilization as two techniques of phenol storage.

PRESERVATION AND RECOVERY OF A MIXED BACTERIA CULTURE

When sewage sludge was inoculated into a buffer (Table 31) supplemented with 500 ppm of phenol, a mixed culture developed, which after several transfers, consisted largely of a Pseudomonas sp. The preservation of this phenolutilizing culture was tested by inoculating 2.8×10^{10} colony-forming units (CFU) of culture into 10-ml solutions of buffer/salts medium with the following additions: (1) no addition, (2) 5% glucose, (3) 5% glutamate, and (4) 5% peptone. Each solution was then divided into two 5.0-ml samples, one for preservation by freezing, the other for preservation by lyophilization. One percent cellulose was added as a stabilizing agent to the cultures to be lyophilized.

TABLE 31. BUFFER-SALTS MEDIUM

Salt	Conc. (mg/1)
$MgSO_4 \cdot 7H_2O$	112.5
$ZnSO_4 \cdot 7H_2O$	5.0
$NaMo\ O_4$	2.5
$KH_2\ PO_4$	680.0
$Na_2H\ PO_4$	700.0
$Ca\ Cl_2$	27.5
$Fe\ Cl_2$	0.5
$NH_4\ Cl$	2600.0

All samples were frozen over a period of 30 minutes in a -20° C freezer. The samples to be test-preserved by freezing were left at -20° C; the samples to be lyophilized were removed from the freezer and placed in dry ice-acetone for 30 seconds, then vacuum-dried overnight, flame sealed, and stored at room temperature. After one week the 5.0-ml frozen samples were thawed and diluted by the addition of 5.0 ml of 0.5% peptone in buffer-salts solution. The freeze-dried samples were rehydrated by the addition of 10.0 ml of 0.5% peptone in buffer-salts. ATP determinations were made at one and at four hours after rehydration or thawing; plate counts of colony-forming units were done at 30 min after removal of the cultures from storage conditions. The results obtained are recorded in Tables 32 through 35.

TABLE 32. SURVIVORS OF FREEZE-STORAGE QUANTITATED BY PLATE COUNTS

Additions	Remaining organisms (CFU/ml)	% Survival CFU/ml survived [1]
None	3.4×10^8	24.0
5% glucose	3.8×10^8	27.0
5% glutamate	2.6×10^8	19.0
5% peptone	3.4×10^5	0.024

[1] Before freezing, 1.4×10^9 colony forming units per ml (CFU/ml) were present.

TABLE 33. SURVIVORS OF FREEZE-STORAGE QUANTITATED BY ATP DETERMINATIONS

Additions	Fg ATP/ml remaining 1 hr. after thawing[1,2]	Fg ATP/ml remaining 4 hr. after thawing[1,2]
None	5.35×10^8	4.71×10^8
5% glucose	5.84×10^8	3.64×10^8
5% glutamate	1.91×10^9	9.17×10^8
5% peptone	6.60×10^7	4.68×10^7

[1] Before freezing 1.4×10^9 Fg ATP/ml were present.

[2] Fg = femtogram (10^{-12}g).

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TABLE 34. SURVIVORS OF LYOPHILIZATION AS DETERMINED BY PLATE COUNTS

Additions	Concentration (CFU/ml)	% Survival [1]
None	$< 10^2$	----
5% glucose	2.3×10^8	16
5% glutamate	1.7×10^8	12
5% peptone	$< 10^4$	----

[1] Before lyophilization 1.4×10^9 colony-forming units per ml were present.

TABLE 35. SURVIVORS OF LYOPHILIZATION AS QUANTITATED BY ATP DETERMINATION

Additions	Fg ATP/ml determined 1 hr after thawing [1]	Fg ATP/ml determined 4 hr after thawing [1]
None	2.41×10^8	8.3×10^7
5% glucose	4.2×10^8	3.4×10^7
5% glutmate	4.3×10^8	5.3×10^8
5% peptone	4.1×10^8	1.9×10^8

[1] Before lyophilization 1.4×10^9 Fg ATP/ml were present.

It was concluded from these data that glucose and glutamic acid both offer some protection against the effects of freezing and lyophilization. Higher survival after lyophilization was achieved by changing culture handling techniques after reconstitution.

Next, the effects of various rehydration media on recovery of preserved phenol-utilizing cultures were examined. In this experiment, multiple ampoules (each containing 5 ml of 5×10^9 CFU/ml phenol-utilizing culture) were lyophilized as previously described and stored one week at room temperature. The cultures in these ampoules were then rehydrated in 10 ml of buffer-salts medium supplemented with either: (1) 100 ppm phenol, (2) 500 ppm phenol, (3) one percent peptone, (4) one percent glutamate, (5) one percent glucose, or (6) no additions. The contents of an additional ampoule were rehydrated in demineralized water only. The rehydrated cultures were then incubated at $25^{\circ}C$ in capped 18-cm (7-in) test tubes with shaking for 3 hrs.

Colony-forming units were determined by dilution of the seven test cultures in buffer-salts medium and plating in triplicate on nutrient agar plates supplemented with 500 ppm phenol. The results obtained are presented in Table 36. These data show little significant difference among the rehydration media tested and indicate that lyophilized phenol-utilizing mixed cultures may be successfully rehydrated in phenol-containing medium.

It was also of interest to investigate the recovery of energy-generating ability by a lyophilized mixed culture of phenol-utilizing microorganisms. A culture that contained 4.7×10^9 Fg* ATP/ml was lyophilized and stored at room temperature as previously described. After one week the vial was opened and the culture rehydrated with 10 ml of 500 ppm phenol in buffer salts. The 10-ml fluid sample was incubated with shaking at 25°C in a capped 18-cm (7-in) test tube and 0.1 ml samples were removed immediately and at intervals of 1.5, 3, 4, and 8 hours for ATP determination in a DuPont Luminescence Biometer. The results of this experiment show an initial drop in ATP concentration followed by a steady rise from 1.5 to 8 hrs. This may indicate that after an early period of recovery, during which energy generation is reduced, ATP formation or cell number rapidly increases.

TABLE 36. THE EFFECTS OF VARIOUS REHYDRATION FLUIDS ON RECOVERY FROM LYOPHILIZATION BY PHENOL-UTILIZING MIXED CULTURES

Additions	Concentration (CFU/ml)
Demineralized water	1.3×10^9
Buffer-salts with:	
No additions	1.3×10^9
100 ppm phenol	1.2×10^9
500 ppm phenol	1.4×10^9
1% peptone	1.7×10^9
1% glutamate	1.0×10^9
1% glucose	1.4×10^9

PRESERVATION AND RECOVERY OF A YEAST CULTURE

A phenol-utilizing yeast strain, Geotrichum candidum, was grown in a defined medium consisting of 0.02 M phosphate, 1.0 g/l NH_4Cl, and small amounts of other inorganic salts supplemented with 500 ppm phenol as the sole carbon source. After 24 hours of incubation, 0.01% yeast extract was added to the culture and incubation was continued for another 24 hours. Cells were harvested by centrifugation, washed, and suspended in a 50% dilution of the mineral-salts growth medium or dilute mineral-salts medium supplemented with 5.0% glucose. Samples of each suspension were frozen and lyophilized. Another set of 0.5-ml aliquots were placed in petri dishes and dried under vacuum without prior freezing. Plate counts were made on samples of the suspension before preservation.

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* Fg= femptogram (10^{12}gm)

Samples were rehydrated in mineral salts medium supplemented with 500 ppm phenol, incubated with shaking for 45 minutes, diluted, and plated on mineralsalts agar supplemented with 500 ppm phenol. Table 37 shows the CFU's in the two circumstances and confirms the suspicion that yeast do not survive lyophilization as well as bacteria.

TABLE 37. SURVIVAL OF PHENOL-UTILIZING GEOTRICHUM[1]

	Lyophilization		Dried from liquid	
	No addition	5% glucose	No addition	5% Glucose
CFU/ml	2.7×10^6	7.3×10^7	2.1×10^6	1.3×10^8
% Survival	0.81	22	0.64	39

[1] Initial count was 3.3×10^8

SECTION II

COUNTERMEASURE APPLICATION

The feasibility of biological countermeasure methods for the removal of phenol and methanol is largely dependent on the amount of acclimated cultures at hand. Phenol and methanol are products or by-products of some manufacturing processes and are inevitably discharged in waste streams. If these wastes are biologically treated, the treatment plants are sources of acclimated cultures. Acclimated bacterial cultures may be stored in a frozen or powdered form in a dormant state for later use (Armstrong et al., 1974). However, these storage problems are beyond the scope of this study and this chapter only investigates the use of acclimated cultures for the actual abatement of phenol and methanol spills. To improve usefulness of the cultures, some auxiliary devices are employed and their mechanisms are studied. Owing to the experimental and time limitations, some of this work is largely theoretical. However, all of these theoretical developments are soundly based on the experimental results or accepted theories, or are directly or indirectly proved by the laboratory-scale tests. For example, the material exchange mechanism through cloth bags is investigated based on the experimental results. Then, the cloth bag efficiency and the substrate removal kinetics using cloth bags are theoretically developed from the material exchange mechanism. Finally, theoretically developed substrate removal kinetics are used to predict the experimental results within a five percent error.

EXPERIMENTAL METHODS

Equipment and Reagents

In addition to the equipment and reagents used in the biological treatability tests, the following chemicals and devices were employed.

Rhodamine B dye was used for the purpose of visual tagging of the material being transported.

A model river was used for material transportation and bulk sludge application tests, tests for material exchange through cloth bags, and confining-barrier application tests. The model river consists of two parallel channels (Figure 54); each is 38.1 cm wide, 45.72 cm deep, and 60.95 m long and has a 10- to 15-cm layer of sediment on the bottom. The bottom sediment was transplanted from Lake Austin in 1962 and has grains with a geometric mean diameter of 0.173 mm and a geometric standard deviation of 1.70 mm (Kludo and Gloyna, 1969). The flow is controlled using a constant-head tank, inlet valves, and V-notched weirs. Water depth is regulated with an adjustable outlet weir. Groundwater is supplied to the model river from a $1.9x10^6$-l supply tank.

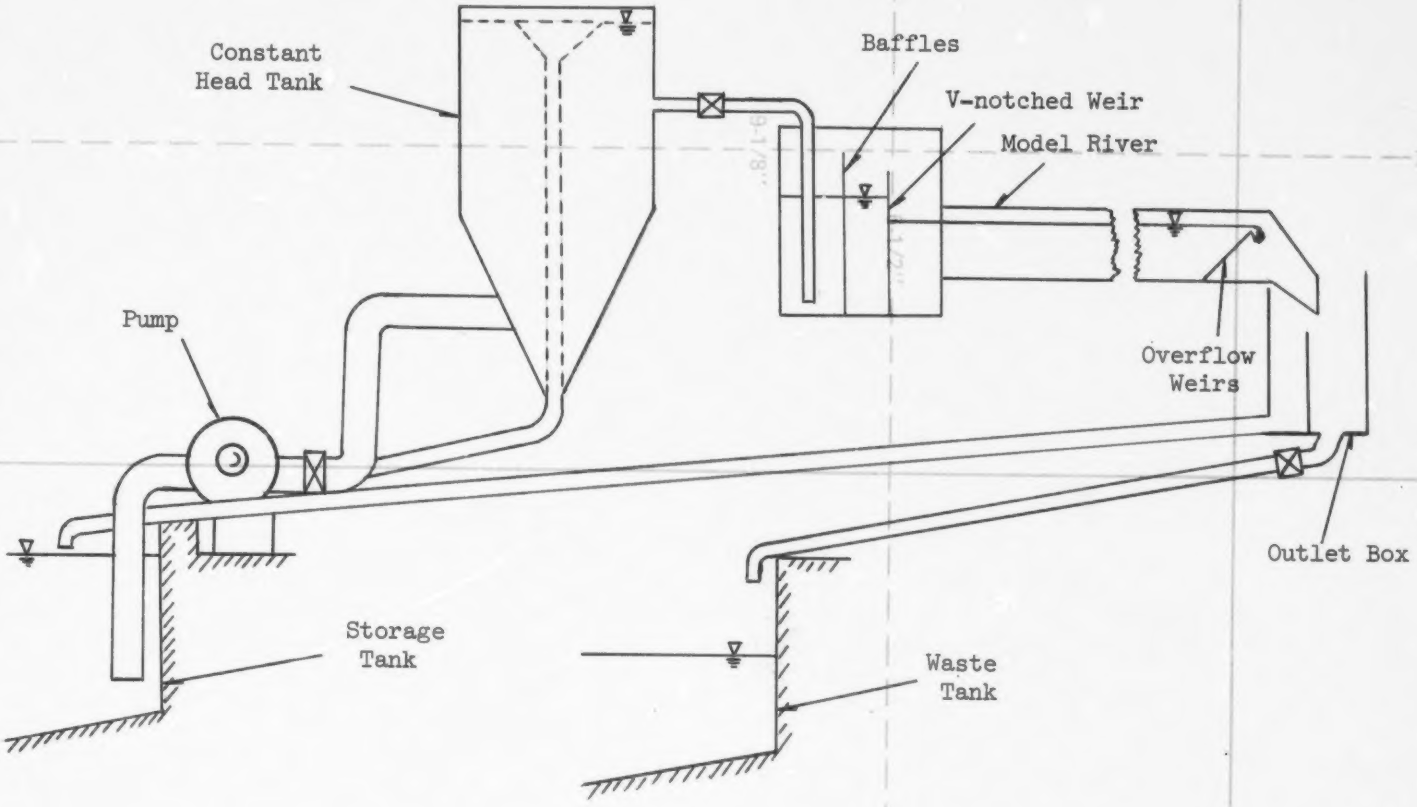
Constant
Head Tank
Baffles
V-notched Weir
Model River
Pump
Overflow
Weirs
Outlet Box
Storage
Tank
Waste
Tank

Figure 54. Model river system.

FIG. 54

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Cloth bags were employed as a means of sludge containment. They were made of cotton cloth with an average pore diameter of about 0.2 mm and were cylindrical in shape with dimensions of 3.18 cm (diameter) and 25 cm (length) and were kept fully expanded with wire-cloth frames. Styrofoam was attached to the top of the bag as a floating aid and had a small hole (about 1 cm diameter) in the middle for the purpose of sludge injection and sampling. Details are shown in Figure 55.

Confining barriers were used to prevent dispersion of spilled materials. They were made of vinyl, were cylindrical in shape, and had a 27-cm diameter and a 40-cm height with an open top and bottom. A wire frame support maintained the cylindrical shape. Styrofoam was attached to the top of the barrier when flotation was desired and weight was applied to the bottom when a fixed barrier was desired. Details are shown in Figure 56.

Procedures

Material Transportation and Application of Bulk Sludge in the Model River --

Phenol and methanol were spilled in the model river with and without acclimated activated sludge. The sludge was sonicated for several seconds to reduce floc size for the purpose of improving sludge floatability. To detect material transport visually, a minute amount of Rhodamine B dye was added to the phenol and methanol. Samples were taken at various distances downstream from the point of spillage at certain time intervals and were analyzed for TOC and VSS. TOC and VSS contributed by the flume water was subtracted from the TOC and VSS measurements to delineate the TOC due to phenol or methanol alone and the VSS contributed by acclimated sludge alone. TOC due to the Rhodamine B dye was negligible. The model river flow was 10.0 liter/min and the water depth was 15.8 cm, giving a 603-cm^2 cross-sectional area and a 0.276-cm/sec velocity.

Material Exchange through Cloth Bags --

Material exchange rates through the cloth bags were measured in batch reactors and in the model river. Elutriated and settled sludge was in-activated with 10 mg/l of silver, using a silver nitrate solution, and was mixed with known amounts of phenol or methanol. A small amount of Rhodamine B dye was added to the sludge to detect the material transport visually and to plan the sampling times.

In batch reactor tests, cloth bags were filled with sludge while slowly immersed into 3-liter reactors. Five reactors were aerated at an air flow of 1 liter air/min/liter water to provide turbulence and five other reactors were maintained in a quiescent condition. These tests were carried out for both phenol and methanol sludge. The water outside the bags was sampled at pre-set time intervals for TOC analysis. The water volume decrease outside the bags caused by sampling was accounted for in the data analysis.

In the model river, the cloth bags were filled with methanol-acclimated sludge prepared in the previously described manner while they were gradually immersed in such a way that the hydrostatic pressure inside the bags did not allow the flume water to flow into the bags. The mixed liquor inside the bags was analyzed for TOC at the end of each test. The test flume velocities were 0.106,0.191, 0.575, 1.551,2.250, and 2.731 cm/sec. Three or four bags were used for each test velocity. Rhodamine B dye solution was used to visually observe the flow regimes.

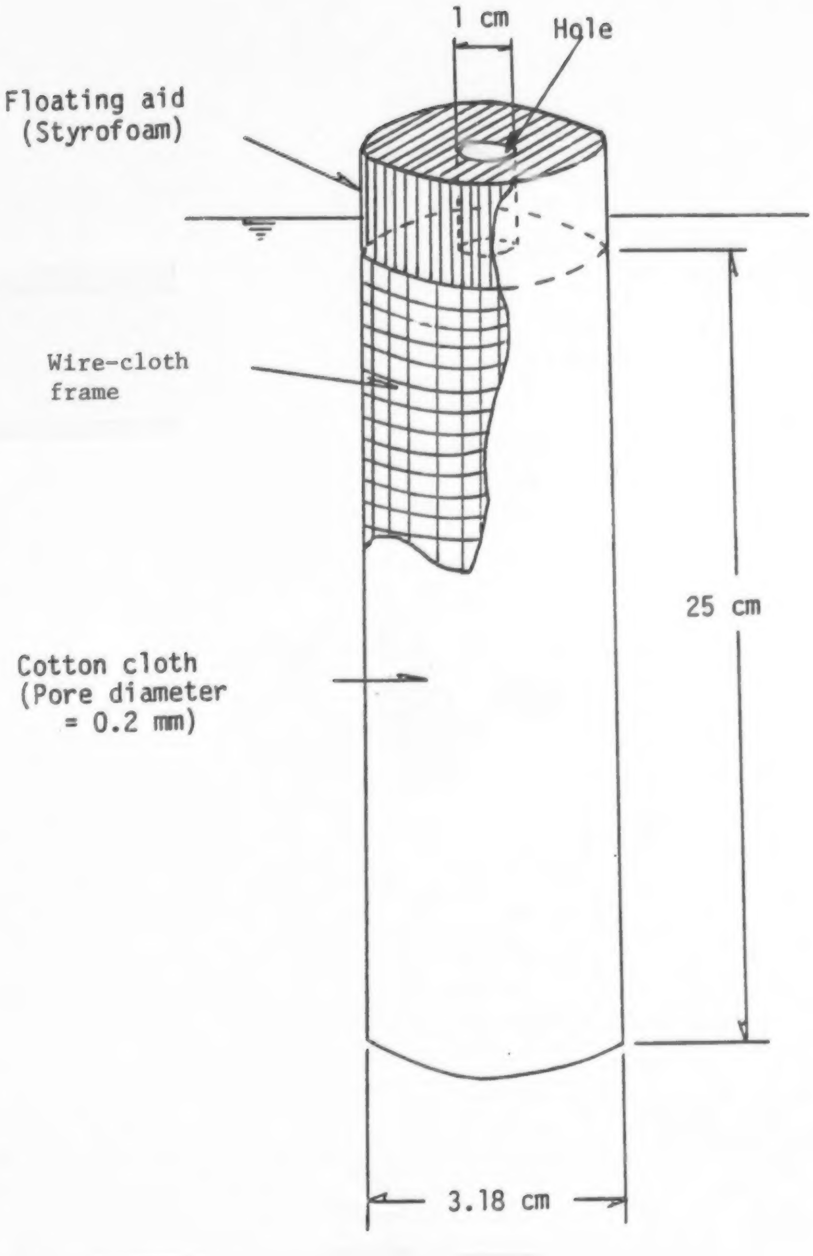
1 cm
Hole
Floating aid
(Styrofoam)
Wire-cloth
frame
25 cm
Cotton cloth
(Pore diameter
= 0.2 mm)
3.18 cm

Figure 55. Details of cloth bag.

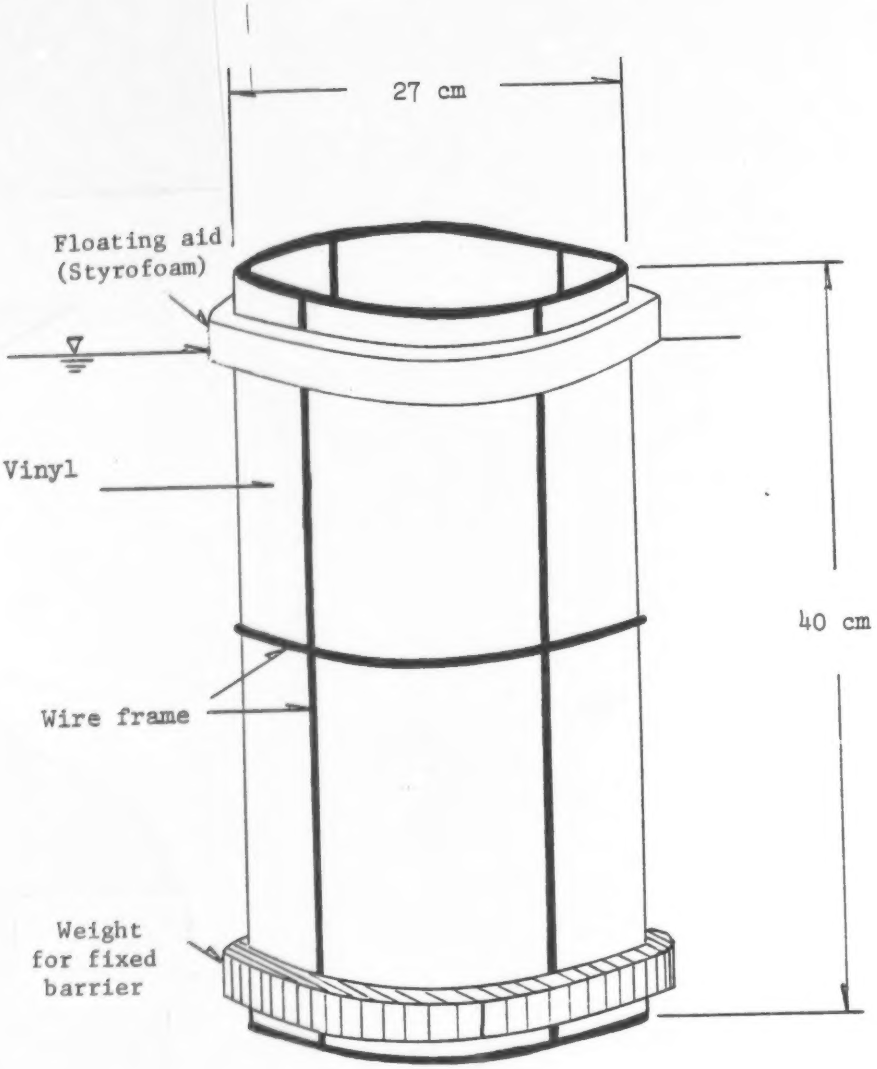
27 cm
Floating aid
(Styrofoam)
Vinyl
40 cm
Wire frame
Weight
for fixed
barrier

Figure 56. Details of confining barrier.

Cloth Bag Application --

Three-liter batch reactors were filled with 30 ml each of Nutrient Solution I and the Mineral Solution. Cloth bags with elutriated settled sludge were immersed in the reactors, which were aerated at room temperature. They were fed with 700 mg/l of methanol (as TOC) and waters outside the bags were sampled at pre-set time intervals for TOC analysis. To calibrate methanol loss by stripping, one reactor was operated without a cloth bag.

Confining Barrier Application

Floating barrier--The model river velocity and depth were adjusted to 0.85 cm/sec and 35.2 cm, respectively. The floating barrier was immersed in the flume so that the clearance between the barrier and the bottom of the flume was less than 3 cm. Rhodamine B dye solution was spilled inside the barrier and color intensity was observed visually. Thirty minutes after the dye spill, the color intensity inside the barrier approached the same level as that outside the barrier. Because of this poor containment efficiency, no further tests were done with the floating barrier.

Fixed barrier--Under the flume conditions described above, phenol and methanol were spilled in the model river. The fixed barriers were dropped at the spill site. Cloth bags with known amounts of acclimated activated sludge were applied inside the barriers and air was supplied. The water outside the bags was sampled and analyzed for TOC. TOC contributed by the flume water was subtracted from the TOC measurements. For the barrier efficiency study, cloth bags and air were not supplied.

ORGANIZATION OF BIOLOGICAL TREATABILITY DATA FOR COUNTERMEASURE DESIGN

The procedure for obtaining information that is needed in setting up countermeasure plans for biological treatability can be summarized as follows.

1 Examine the characteristics of water to be treated (spill concentration, pH, alkalinity, salinity, temperature etc.).

2. From Figure 20 and Table 10, or Figure 31 and Table 16, estimate the equivalent pH for treatment or average pH and efficiency of dilution water if no chemical aids will be used.

3. From iso-f diagrams at the nearest temperature, Figures (10, 11, 12, 23, 24, or 25) evaluate f corresponding to the given pH and salinity. Then,

$$k_{T_o} = f\ k_{(pH-7,\ sal =\ ppt)}^{x}$$

is the efficiency of dilution water.

4. From iso-θ diagrams (Figs, 13, 26, or 27), find θ. Then, $k_T = k_{T_o}\,\theta^{(T-T_o)}$.

5. Evaluate k_d from $k_d = 0.066\ k^{0.87}$ for phenol and from $k_d = 0.0115\ k^{0.634}$ for methanol.

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6. Evaluate b' from $b' = 1.42\ k_d$.

7. Constant values used are $a = 1.21$, $K_s = 236$ mg/l, and $a' = 1.39$ for phenol and $a = 1.25$, $K_s = 2{,}330$ mg/l, and $a' = 2.23$ for methanol.

The above six coefficients are specific for the wastes to be treated. The following sections show how they are utilized in designing countermeasures for spills of phenol and methanol.

PORTABLE TREATMENT SYSTEMS

Design of CSTR System

Continuous-stirred-tank-reactor (CSTR) systems are widely used in the treatment of continuous waste input and can be a possible solution when portable treatment facilities are brought to a spill site. Essential parts of CSTR systems are shown in Figure 57.

In a CSTR system, the mass accumulation in the reactor can be expressed as:

$$V\ dS/dt = (QS_o - QS_e) - \left(\frac{kXS_e}{K_s + S_e} + k_e S_e\right) V \qquad (76)$$

where: Q = flow rate (L^3/T),
V = reactor volume (L^3),
S_o = influent substrate concentration (M/L^3), and
S_e = effluent substrate concentration (M/L^3).

At steady state ($ds/dt=0$) the total biomass required to achieve substrate reduction from S_o to S_e may be found from:

$$VX = \frac{\left\{Q(S_o - S_e) - (k_e V S_e)(K_s + S_e)\right\}}{k S_e} \qquad (77)$$

$$V \frac{dX}{dt} = QX_o - (Q - Q_w)X_e + aQ(S_o - S_e - k_e S_e \frac{V}{Q}) - k_d VX - Q_w X_w, \qquad (78)$$

where: X_o = influent biomass concentration (M/L^3),
X_e = effluent biomass concentration (M/L^3),
Q_w = sludge waste flow rate (L^3/T), and
X_w = biomass concentration in sludge waste flow (M/L^3).

If influent and effluent solids are negligible, then:

$$\frac{dX}{dt} = a \frac{Q}{V}(S_o - S_e) - a\, k_e S_e - k_d X - \frac{Q}{V}w\, X_w\,. \qquad (78')$$

At steady state ($\frac{dX}{dt} = 0$) the sludge waste required to achieve the total biomass (VX) in the reactor is:

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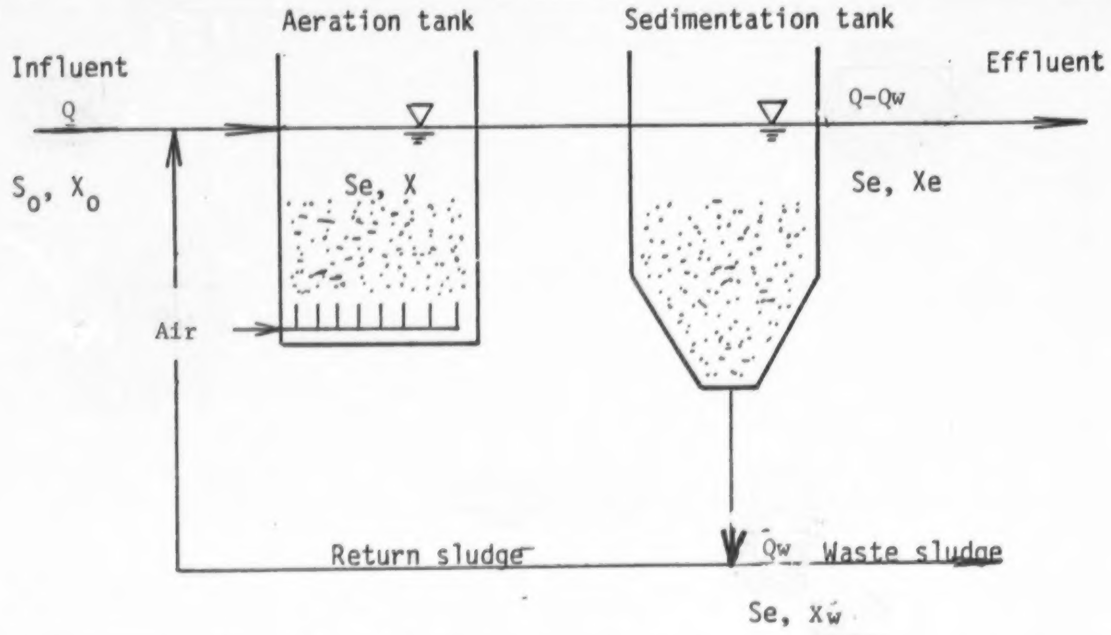

Figure 57. Essential parts of a CSTR system.

$$Q_wX_w = a\{Q(S_o-S_e) - k_eVS_e\} - k_dVX. \qquad (79)$$

The maximum achievable total biomass in the reactor is: $a/k_d\ \{Q(S_o - S_e) - k_eVS_e\}$, which is obtained when no sludge is wasted. In order to prevent sludge washout, sludge waste should be less than $a\{Q(S_o - S_e) - k_eS_eV\}$ (see Figure 58).

The total oxygen requirement at steady state is:

$$Rr\ V = a'Q(S_o - S_e) - k_eS_eV - b'XV\ . \qquad (80)$$

Design of Batch Treatment System

Batch treatment systems do not necessarily require sophisticated facilities. Any containers can be turned into batch reactors, if it is necessary. Another advantage of batch treatment systems may be that the effluent quality can be controlled very easily. Thus, batch treatment methods offer a highly promising solution for abatement of phenol and methanol spills.

Batch systems can be designed using a numerical method. The time required to reduce the substrate concentration from S_{ii-1} to S_i and the biomass concentration at an i-th station are obtained from Equations 62 and 69, respectively, or:

$$t_i^{(n)} = t_{i-1} + 2\,\frac{a(S_{i-1} - S_i) - (X_i^{(n-1)} - X_{i-1})}{ak_e(S_{i-1} + S_i) + k_d(X_{i-1} + X_i^{(n-1)})} \qquad (81)$$

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and

$$t_i = t_{i-1} + 2\,\frac{a(S_{i-1}-S_i)-(X_i-X_{i-1})}{k_d(X_{i-1}+X_i)}\,. \qquad (84)$$

Since X_i is directly solved from S_i without the aid of t_i, there is no need for iteration when Equations 83 and 84 are concerned.

Equations 81 and 84 are based on the approximation,

$$k_d\int_{t_{i-1}}^{t_i} X\,dt = 1/2\; k_d\,(X_{i-1}+X_i)(t_i-t_{i-1})\,.$$

Therefore, the S increment, $(S_i - S_{i-1})$, should be selected so that it produces very small relative changes in X, calculated as $(X_i-X_{i-1})/X_{i-1}$. When the S increment was satisfied $\left(|(S_i-S_{i-1})/X_{i-1}| \le 1/200\right)$ and $\left(|(S_i-S_{i-1})/S_{i-1}| \le 1/10\right)$ stable results were produced.

Computation results were utilized in developing the batch kinetic diagrams for phenol for k values of 0.01892, 0.02729, 0.3934, 0.05674, and 0.07239 hr^{-1} at dilute and concentrated VSS concentrations as shown in Figures 59-1 to 59-10. The following example explains the use of these diagrams.

Example --

Problem--500 mg/l TOC of phenol waste is to be treated in a batch reactor under environmental conditions that give $k = 0.0645\ hr^{-1}$. What is the aeration time required to obtain 99% phenol removal and what will be the final biomass concentration if 2,000 mg/l of VSS is available initially?

Answer--Use the batch kinetic diagrams that bracket the k value to be used. Draw VSS lines starting from 500 mg/l TOC and 2,000 mg/l VSS in diagrams for $k = 0.05674\ hr^{-1}$ and $k = 0.07239\ hr^{-1}$ (Figures 59-8 and 59-10). Then, read the VSS's at 5 mg/l TOC and the times of the intersecting points of TOC and VSS lines at the initial and final points.

At $k = 0.05674\ hr^{-1}$: (1) VSS at 5 mg/l TOC = 2,460 mg/l, (2) time at 500 mg/l TOC = - 6.1 hours, (3) time at 5 mg/l TOC = 5.8 hours, and (4) time required for 99% removal = 5.8 - (-6.1) = 11.9 hrs.

At $k = 0.07239\ hr^{-1}$: (1) VSS at 5 mg/l TOC = 2,460 mg/l, (2) time at 500 mg/l TOC = -5.0 hours, (3) time at 5 mg/l TOC = 4.5 hours, and (4) time required for 99% removal = 4.5 - (-5.0) = 9.5 hrs.

Then, the time required for 99% phenol removal at $k = 0.0645\ hr^{-1}$ is, by interpolation,

$$9.5 + (11.9-9.5)\,\frac{0.07239-0.0645}{0.07239-0.05674} = 10.7 \text{ hrs.}$$

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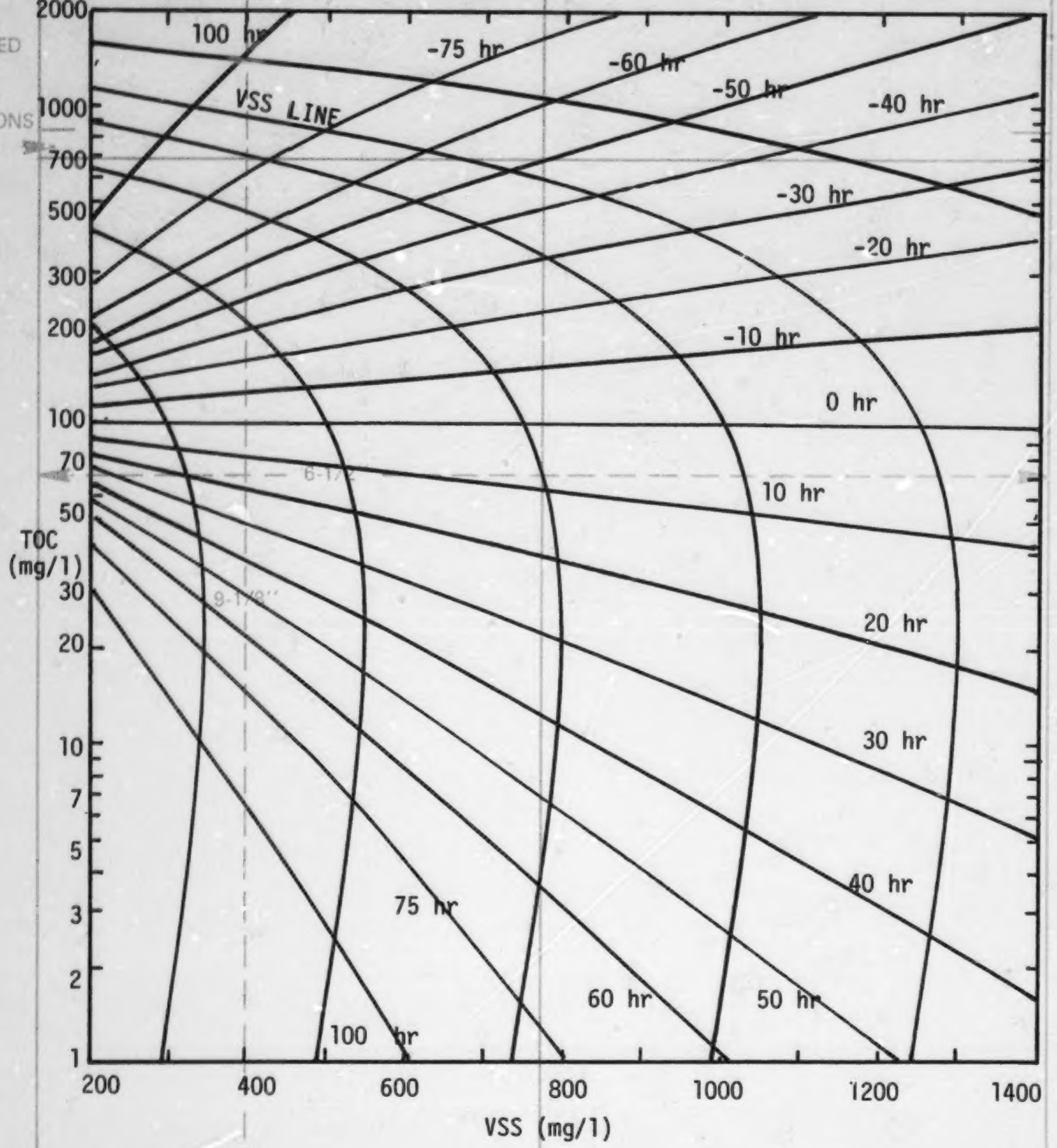

Figure 59-1. Batch kinetic diagram for phenol for dilute VSS at k = 0.01892 hr^{-1}.

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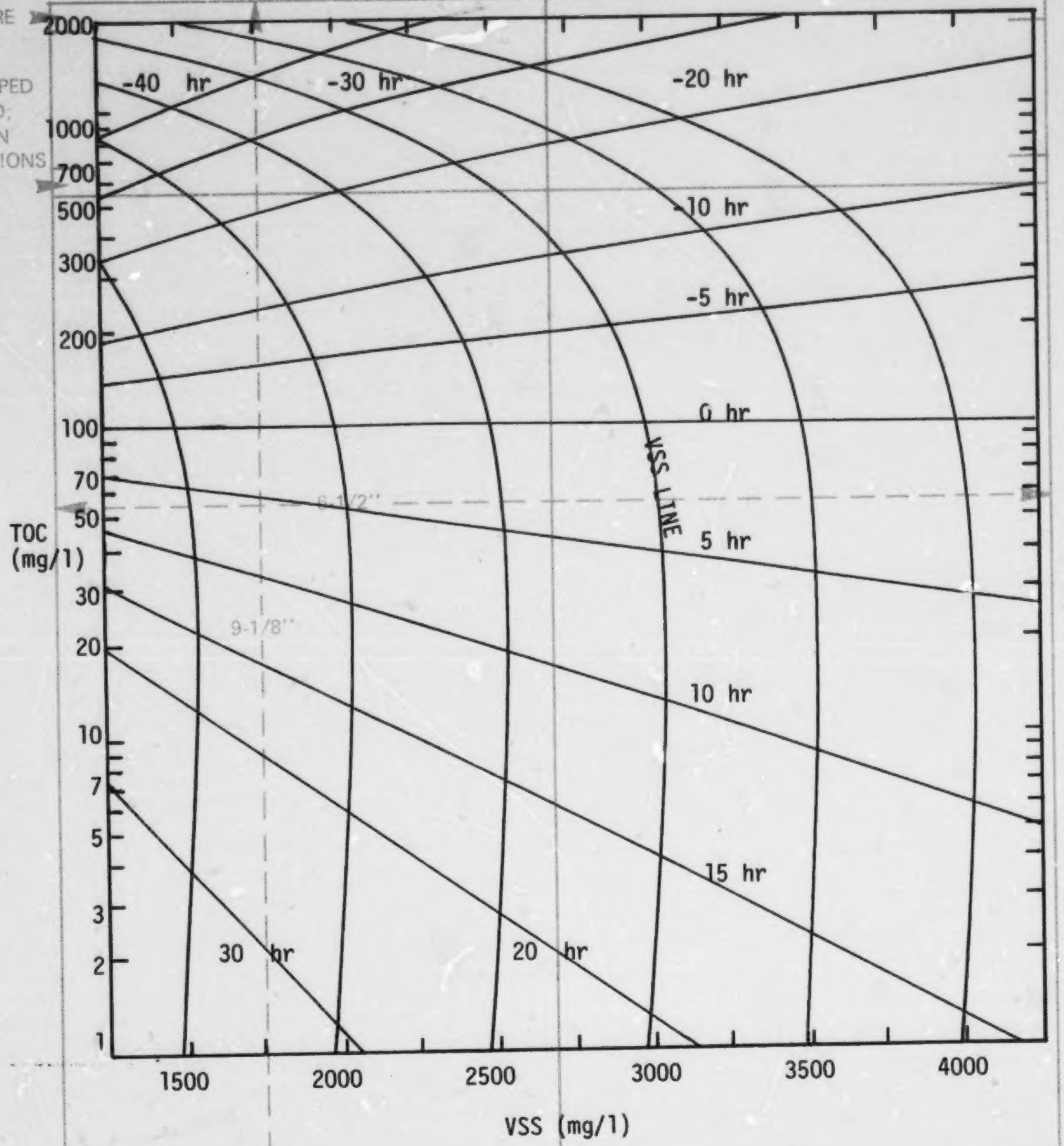

Figure 59-2. Batch kinetic diagram for phenol for concentrated VSS at k = 0.01892 hr^{-1}.

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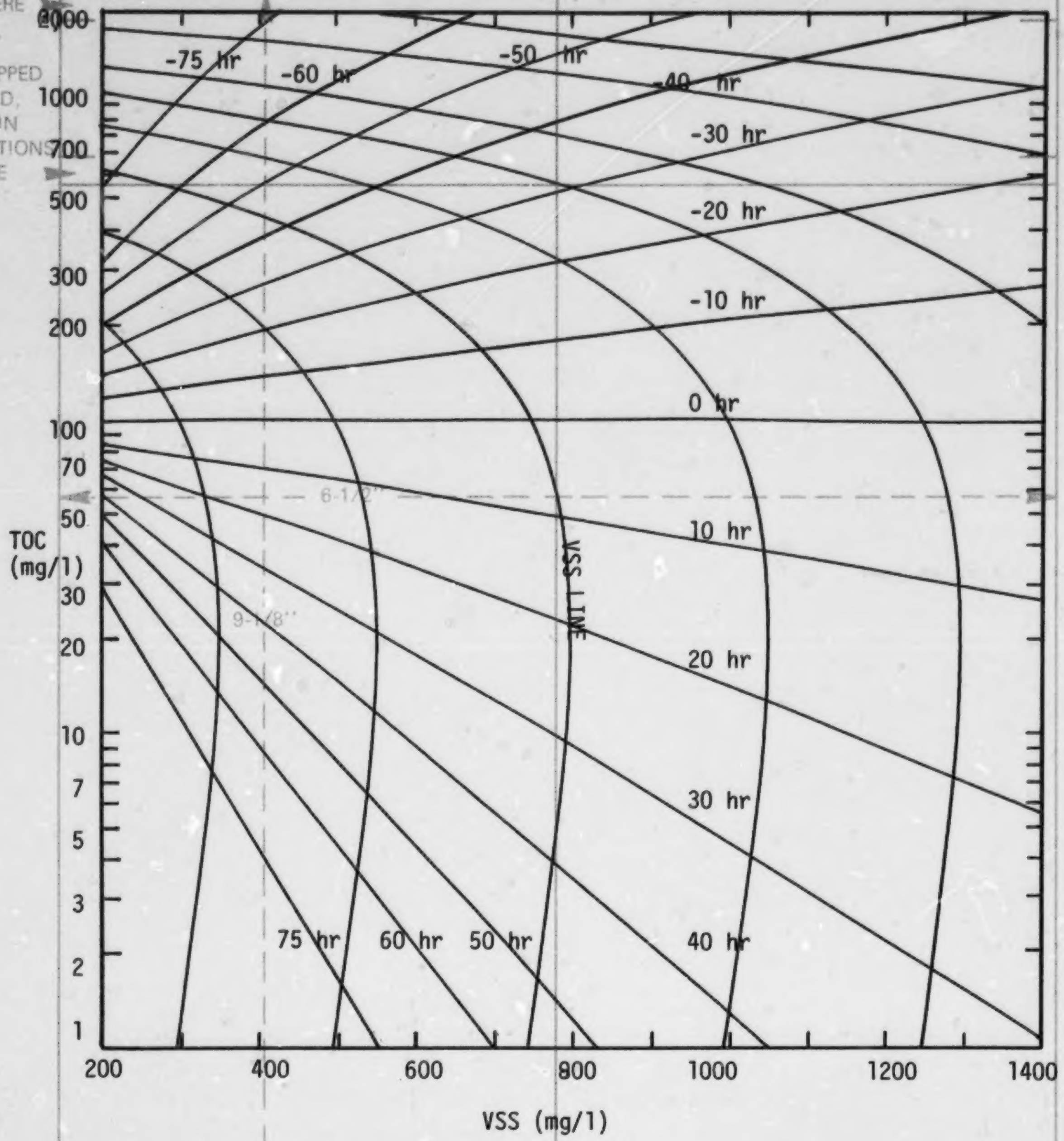

Figure 59-3. Batch kinetic diagram for phenol for dilute VSS at K - 0.02729 hr^{-1}.

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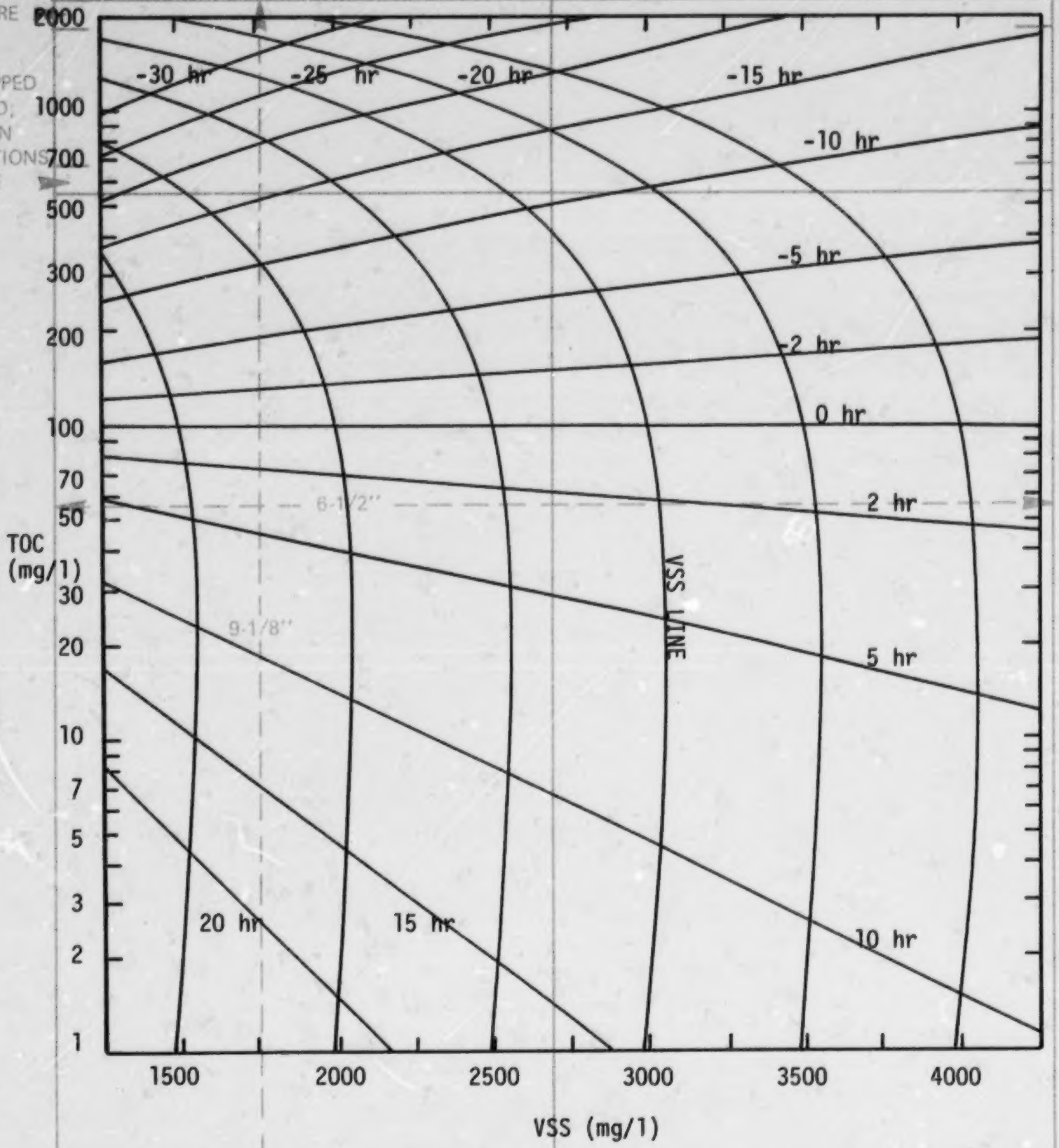

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Figure 59-4. Batch kinetic diagram for phenol for concentrated VSS at $k = 0.02729\ hr^{-1}$.

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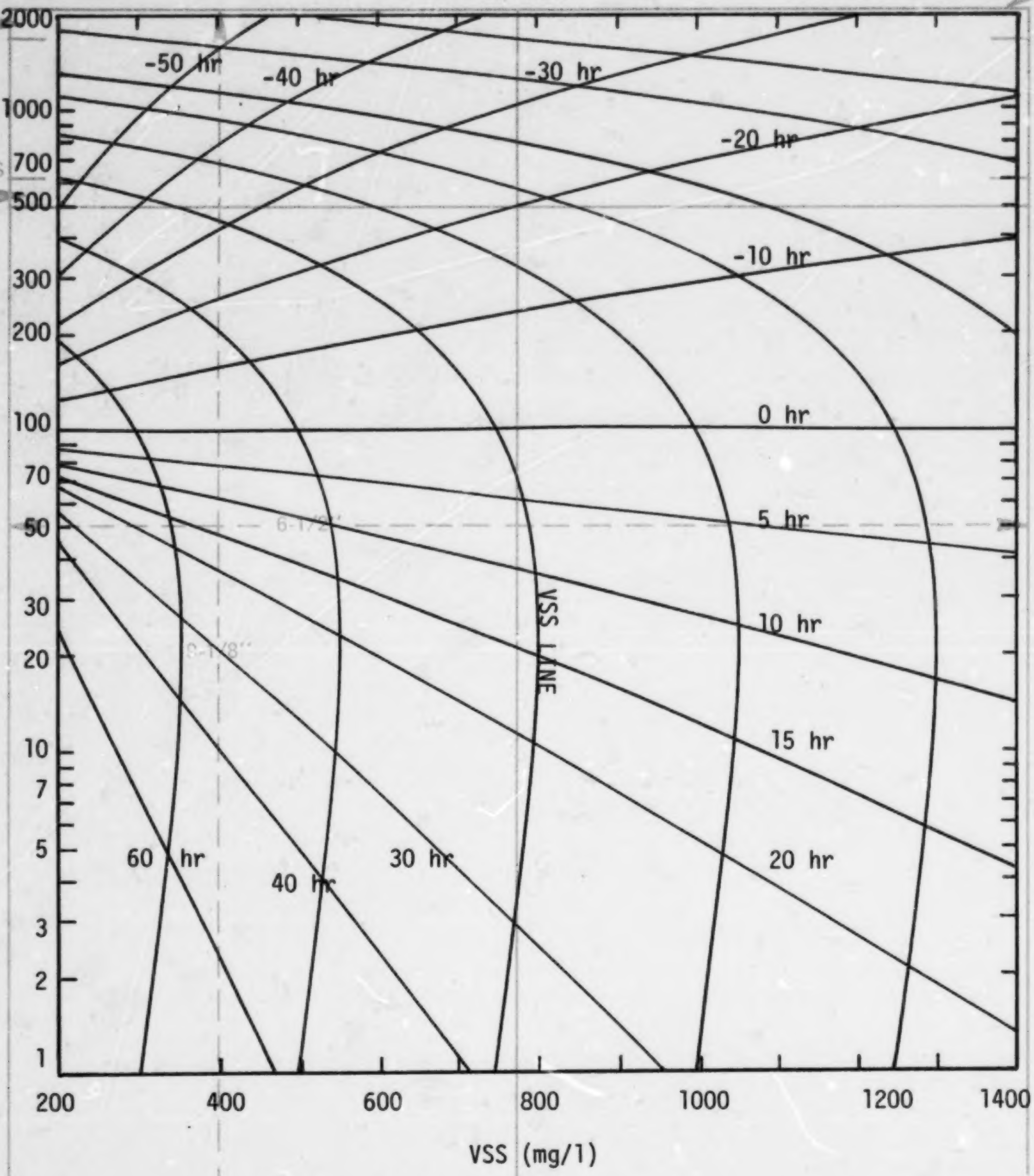

Figure 59-5. Batch kinetic diagram for phenol for dilute VSS at k = 0.03934 hr^{-1}.

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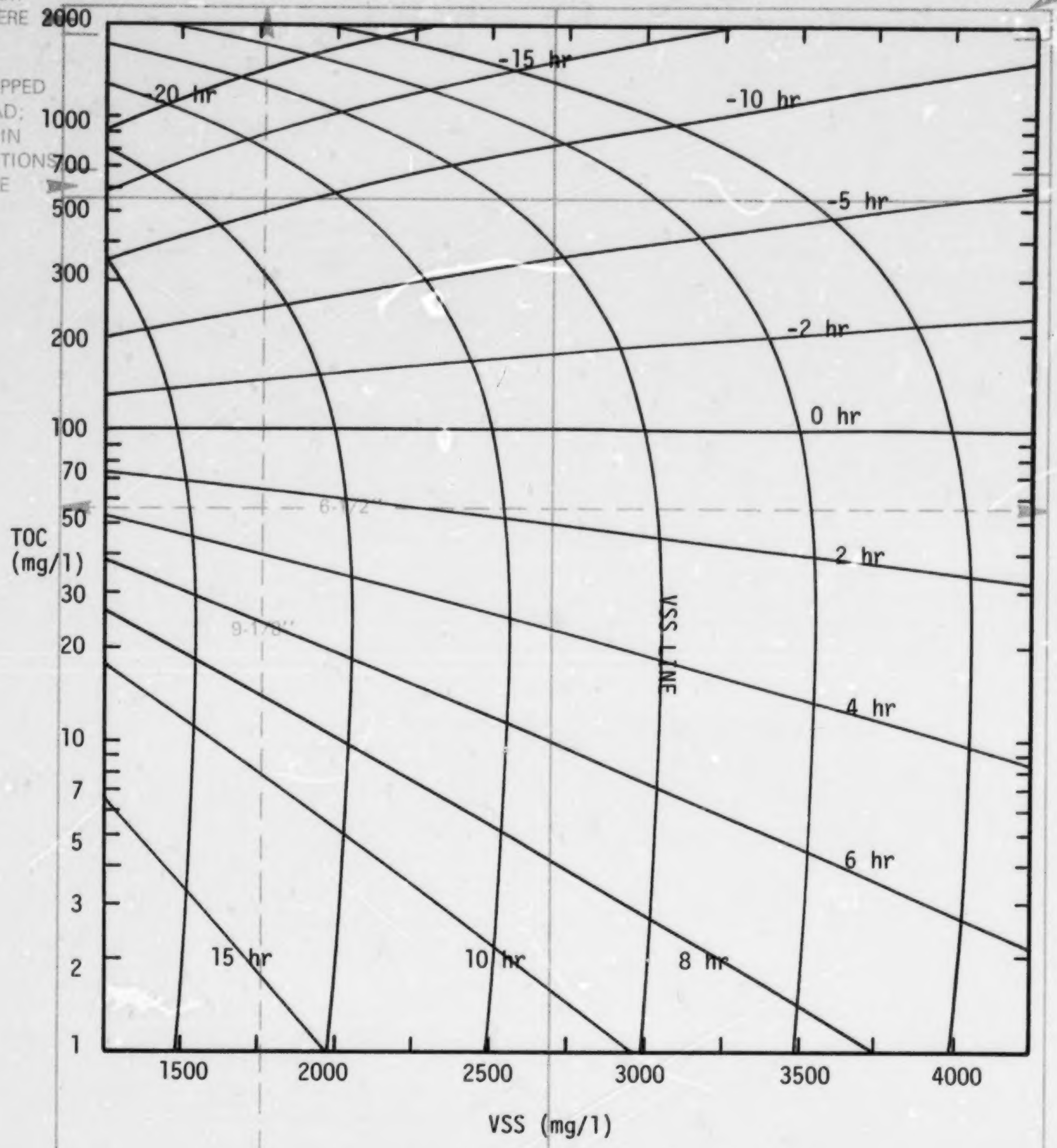

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Figure 59-6. Batch kinetic diagram for phenol for concentrated VSS at $k = 0.03934\ hr^{-1}$.

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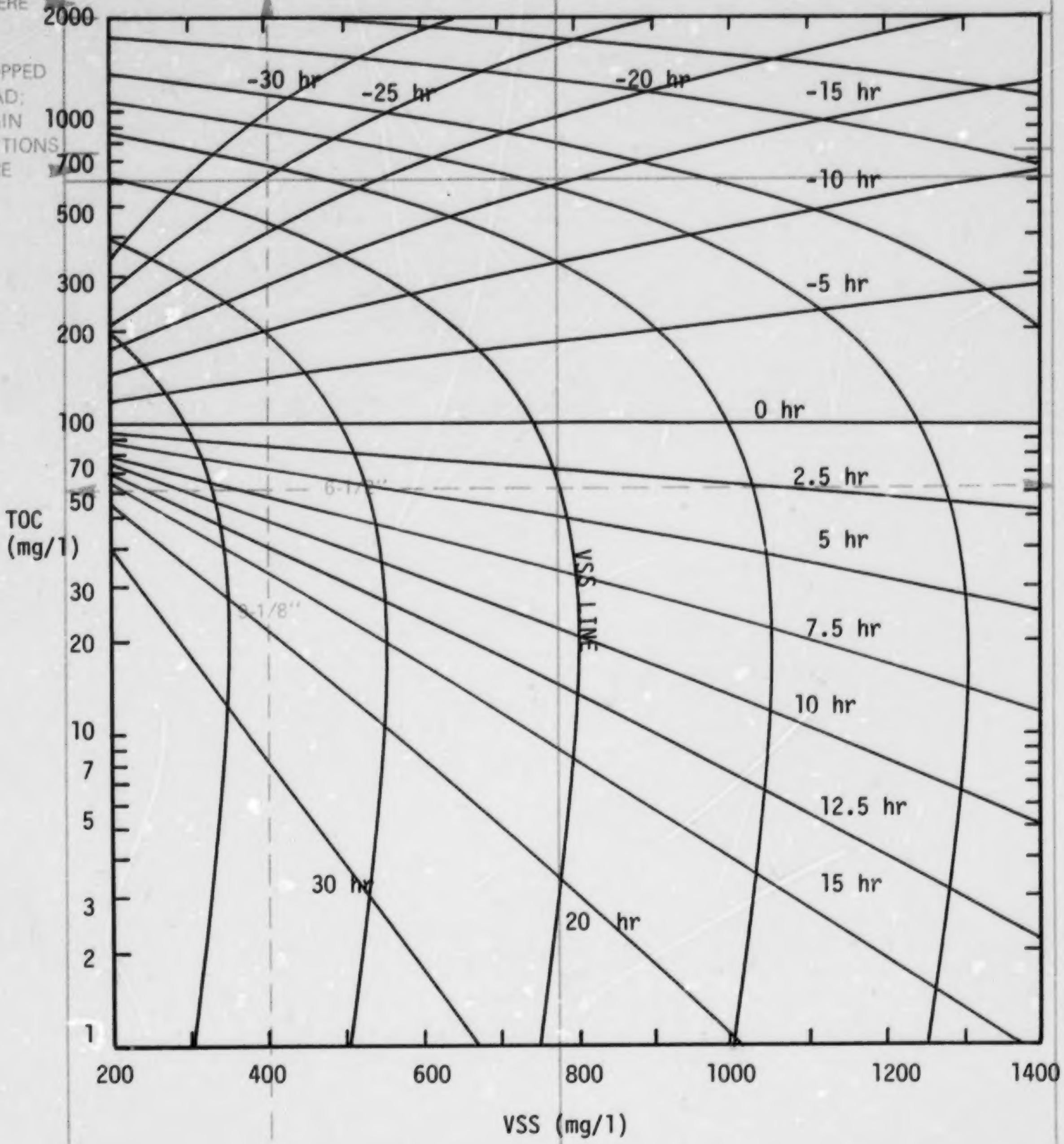

Figure 59-7. Batch kinetic diagram for phenol for dilute VSS at k = 0.05674 hr^{-1}.

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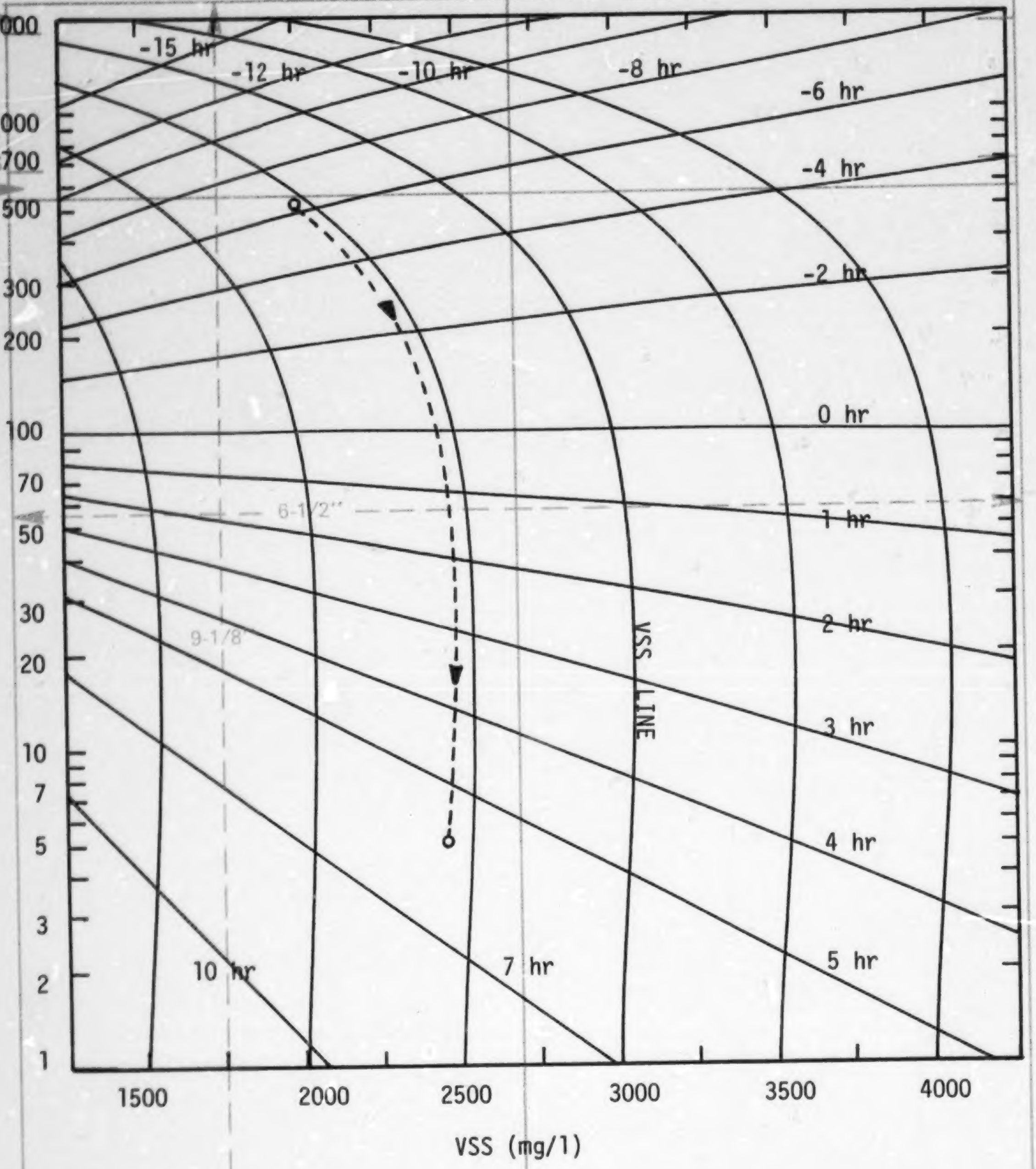

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Figure 59-8. Batch kinetic diagram for phenol for concentrated VSS at k = 0.05674 hr^{-1}.

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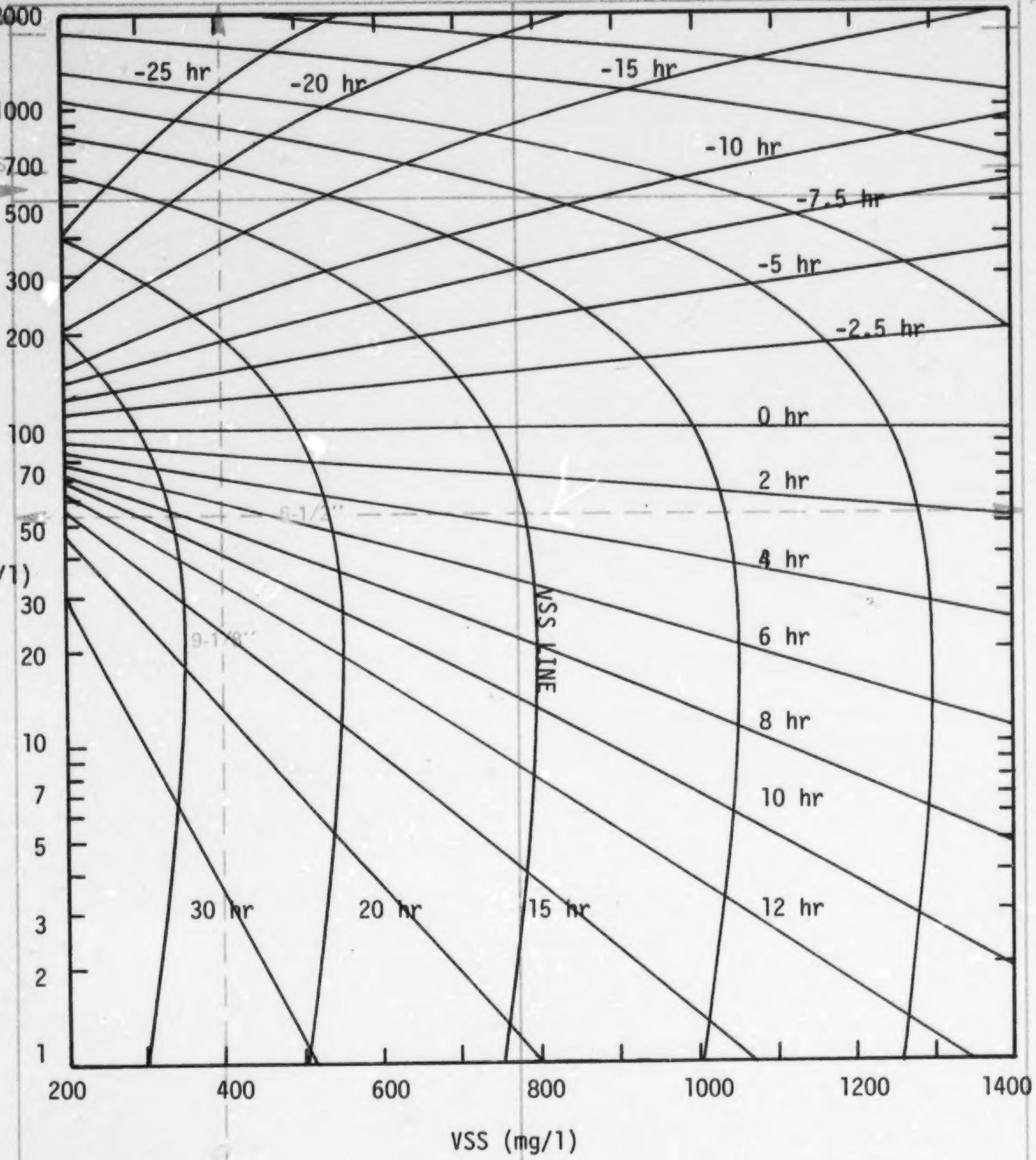

Figure 59-9. Batch kinetic diagram for phenol for dilute VSS at k = 0.07239 hr^{-1}.

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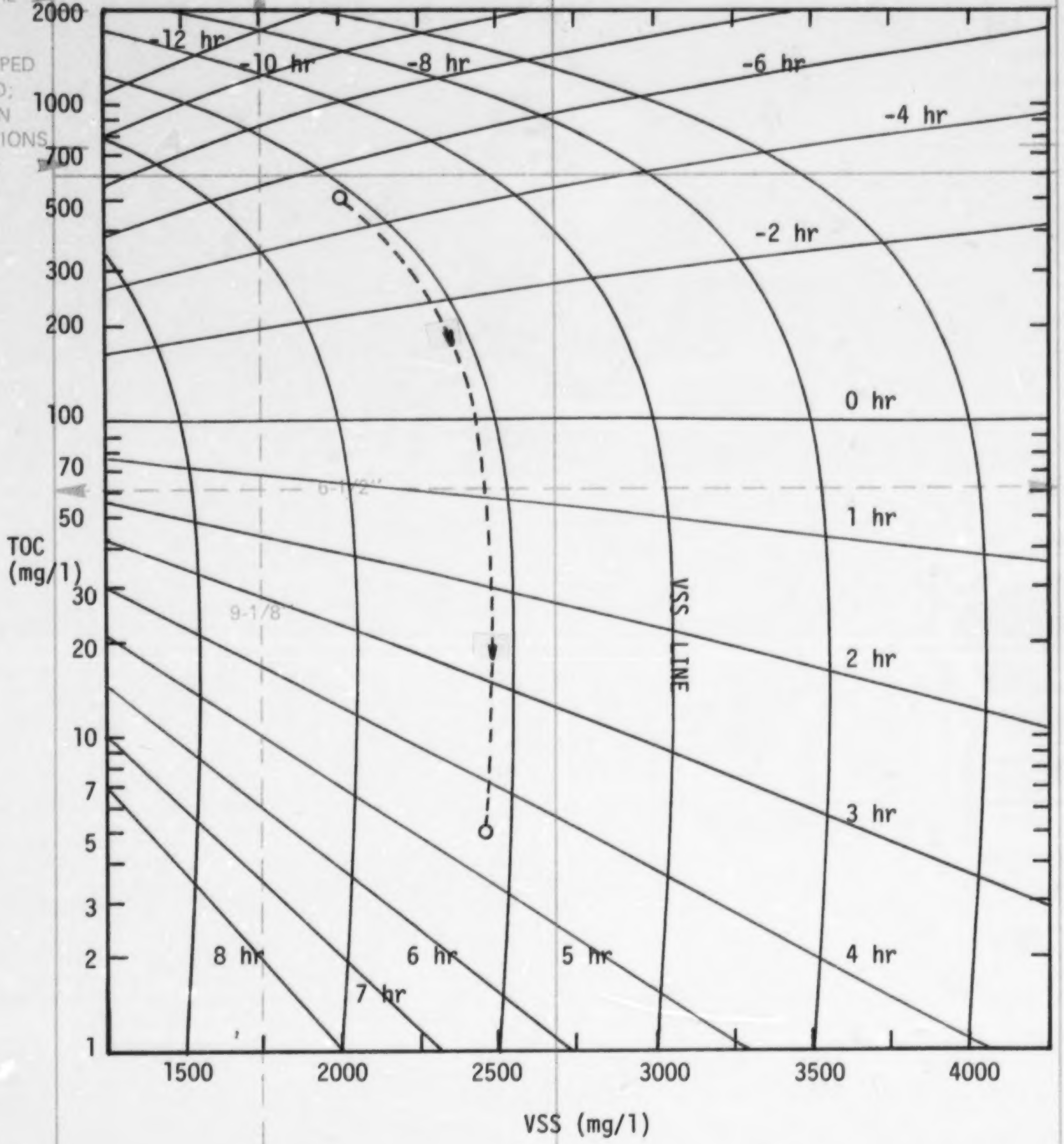

Figure 59-10. Batch kinetic diagram for phenol for concentrated VSS at k = 0.07239 hr^{-1}.

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When three hours of aeration lag time by initial lag phase are taken into consideration, the total aeration time required will be about 14 hours and the final VSS concentration will be 2,460 mg/l.

Oxygen requirements for this system change with time. S and X at a given time are obtained from the batch kinetic diagrams and oxygen requirements at the time are solved from Equation 58 or 73.

Comparison of CSTR and Batch Systems

The biological decomposition rate in the CSTR system is $kXS_e/(K_s+S_e)$ and in the batch system is $kXS/(K_s+S)$. Changing the fractions to a common denominator, one gets $((kX(K_s S_e + S S_e))/((K_s+S_e)(K_s+S))$ for the CSTR system and $((kX(K_s S + SS_e))/((K_s+S_e)(K_s+S))$ for the batch system. Since S in the batch reactor is always greater than or equal to the design effluent quality, S_e, the removal rate in the batch reactor is always better than that in the CSTR.

When there is no stripping loss, the hydraulic detention time τ_c, required to achieve effluent quality, S_e, in the CSTR is:

$$\tau_c = \frac{V}{Q} \quad \frac{(S_o - S_e)(K_s + S_e)}{k X S_e} \tag{85}$$

In the batch reactor, τ_b is solved from Equations 83 and 84. Let the relative efficiency of the CSTR, r, denote the ratio of $_b$ to $_c$, or:

$$r = \tau_b / \tau_c \,. \tag{86}$$

The relative efficiency of the CSTR turned out to be mainly dependent on the influent to effluent quality ratio, S_o/S_e. As influent concentration increases the relative efficiency of CSTR sharply decreases. If an effluent quality of 5 mg/l TOC of phenol is required with 3,000 mg/l of initial VSS available, the efficiency is 0.7 for 10 mg/l influent, 0.27 for 50 mg/l influent, and 0.05 for 500 mg/l influent (see Figure 60). Thus, for highly concentrated spills, the batch systems can be considered as a necessity rather than as a preference.

Biological countermeasures for accidental spills may require as much acclimated activated sludge as is available. Batch systems also have an advantage over CSTR systems in this respect. The sludge production rate in a CSTR, Pr_c, is:

$$Pr_c = a\frac{V}{\tau_c}(S_o - S_e) - a k_e V S_e - k_d V X \tag{87}$$

and that in a batch reactor, Pr_b, may be expressed as:

$$Pr_b = a\frac{V}{\tau_b}(S_o - S_e) - \frac{a k_e V}{\tau_b}\int_0^{\tau_b} S\,dt - \frac{k_d V}{\tau_b}\int_0^{\tau_b} X\,dt \,. \tag{88}$$

If the stripping loss and endogeneous respiration (second and third terms of the equations) are neglected, the ratio of sludge production rate in a CSTR to that in a batch reactor becomes τ_b/τ_c, which is identical to the relative efficiency of a CSTR.

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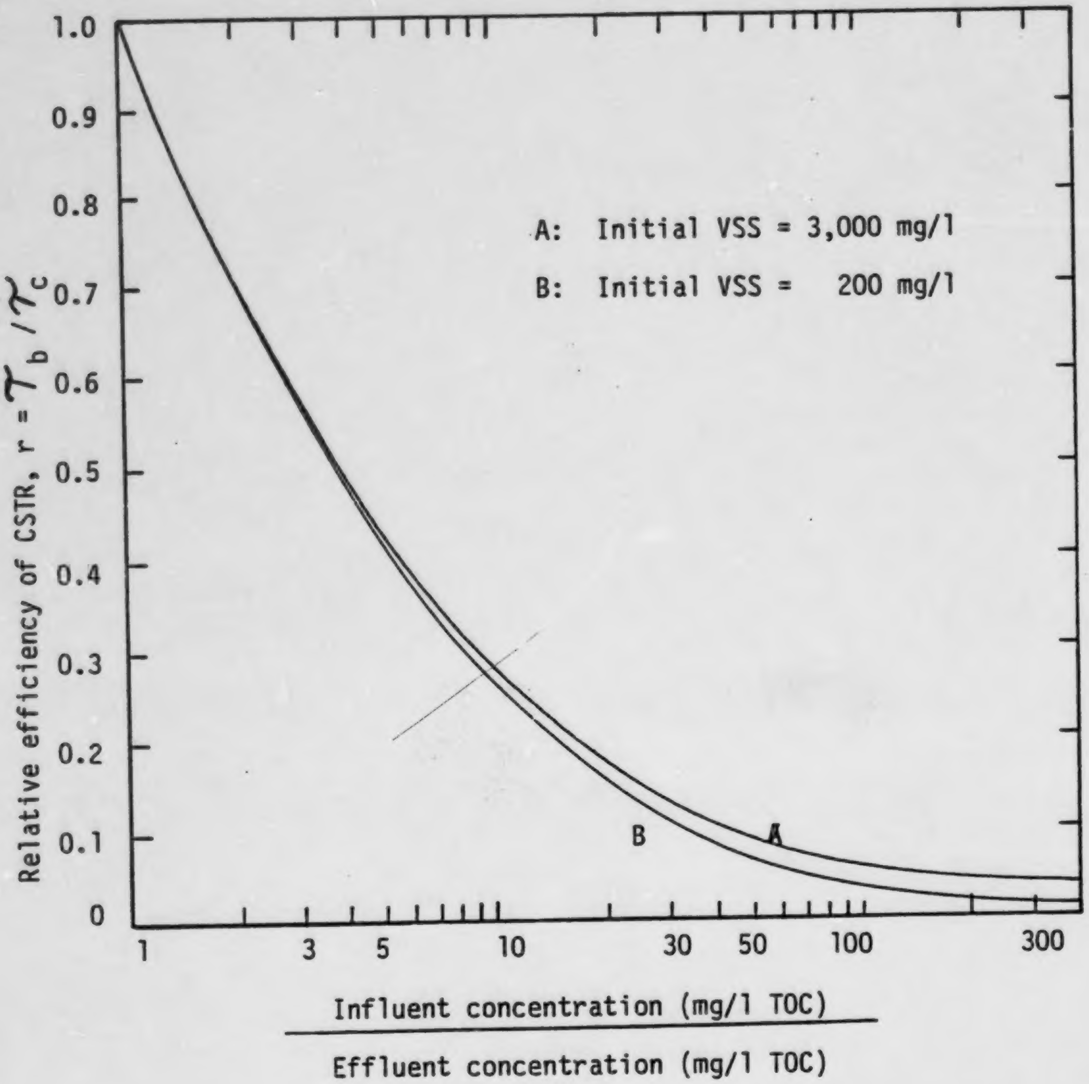

Figure 60. Relative efficiency of CSTR in phenol removal (for effluent quality of 5 mg/l TOC).

Therefore, batch systems produce more sludge.

The following may be considered as disadvantages of batch systems:

1. Batch systems are more susceptible to shock loading.

2. Initial lag periods need to be considered.

3. Rapid substrate removal may cause an unfavorable pH range.

4. Sludge production is a nuisance when it is to be disposed.

5. When consecutive batch treatments are required, there are time losses between each batch, owing to the draining and filling of the reactors and the sludge return.

Methanol-acclimated activated sludge turned out to be highly dispersive and this may impose a serious problem in sludge return when there is no special treatment. Sludge-containing cloth bags can be an easy solution for the sludge return. They can curtail considerable time loss between each batch treatment. Functions of cloth bags are discussed in detail later in this chapter.

IN SITU APPLICATIONS OF THE BIOLOGICAL COUNTERMEASURE

Application of Bulk Sludge in One-Dimensional Dispersion System

When spills occur in rivers and the use of portable treatment facilities is restricted, bulk sludge application methods may be a solution for the treatment of spills. As usually practiced, the dispersion in rivers may be considered one-dimensional.

The one-dimensional dispersion equation is expressed as:

$$\frac{\partial S}{\partial t} + u\frac{\partial S}{\partial x} = D\frac{\partial^2 S}{\partial x^2}, \qquad (89)$$

where: S = concentration at a distance x and time t (M/L^3),
u = mean flow velocity (L/T), and
D = dispersion coefficient (L^2/T).

For a point source injection of a substance, the concentration distribution has been solved to be (Taylor, 1954; Harris, 1963):

$$S = \frac{M_s}{A_r\sqrt{4\pi Dt}} \exp\left\{-\frac{(x-ut)^2}{4Dt}\right\}, \qquad (90)$$

where: M_s = total mass of substance injected (M) and
A_r = the cross-sectional area of channel (L^2).

Fischer (1966) reported that the mechanism of initial diffusion, characterized by tail effects, was markedly different from that described by Equation 90, so that Equation 89 was the only effective formulation after the initial period. However, phenol and methanol transport tests did not show any evidence of Fischer's theory. The dispersion coefficients were estimated using the Fischer's moment method:

$$D = \frac{1}{2}\frac{\Delta \sigma_\xi^2}{\Delta t} \qquad (91)$$

where:

$$\sigma_\xi^2 = \frac{\int_{-\infty}^{\infty} S\xi^2 \, d\xi}{\int_{-\infty}^{\infty} S \, d\xi} \quad \text{and} \qquad (92)$$

$$\xi = x - ut . \qquad (93)$$

Thus, the dispersion coefficients estimated were not affected by the initial diffusion mechanism, if there was any. The computed concentration distributions from Equation 90 satisfactorily agreed with those observed (Figures 61 and 62). The skewness of the distributions is thought to be attributed to the winds rather than to the initial mixing mechanism. The model river ran from north to south and a southerly wind prevailed throughout the test periods. Test flow velocities could not overcome the winds and the surface water flow was largely governed by winds. During the phenol transport tests, there was a temporary northerly wind, which gradually changed to a mild southerly wind. Thus, there were reverse tail effects in the beginning, which is contrary to Fischer's theory. When the flow rates were extremely small, phenol sank to the bottom, reproducing the so-called tail effect, and methanol floated with arbitrary distributions governed by the winds. Therefore, Equation 90 is thought to be effective in prototype channels on which the winds have little effect.

When dispersive acclimated activated sludge is deployed over the spills according to the pollutants dispersion model, the distribution function of both sludge and pollutants may be approximated as Equation 90. Then, the total mass removal rate can be expressed as:

$$\frac{dM}{dt} = -\int_{\infty}^{\infty} \frac{A_r k X S}{K_s + S}\, dx - \int_{-\infty}^{\infty} A_r k_r S \, dX , \qquad (94)$$

where k_r is the pollutant removal rate coefficient (time^{-1}) contributed by volatilization and bottom sorption, etc, (other than biological reactions). Equation 94 holds true only when there is enough oxygen supply. In most streams, aeration is restricted and the bulk sludge application method cannot be efficient for highly concentrated spills. When the spilled material concentration at peak point is smaller than K_s and when A_r is constant, Equation 94 may be simplified to:

$$\frac{dM_s}{dt} = -\frac{A_r k}{K_s}\int_{-\infty}^{\infty} XS \, dX - \int_{\infty}^{\infty} A_r k_r S \, dX \qquad (94^1)$$

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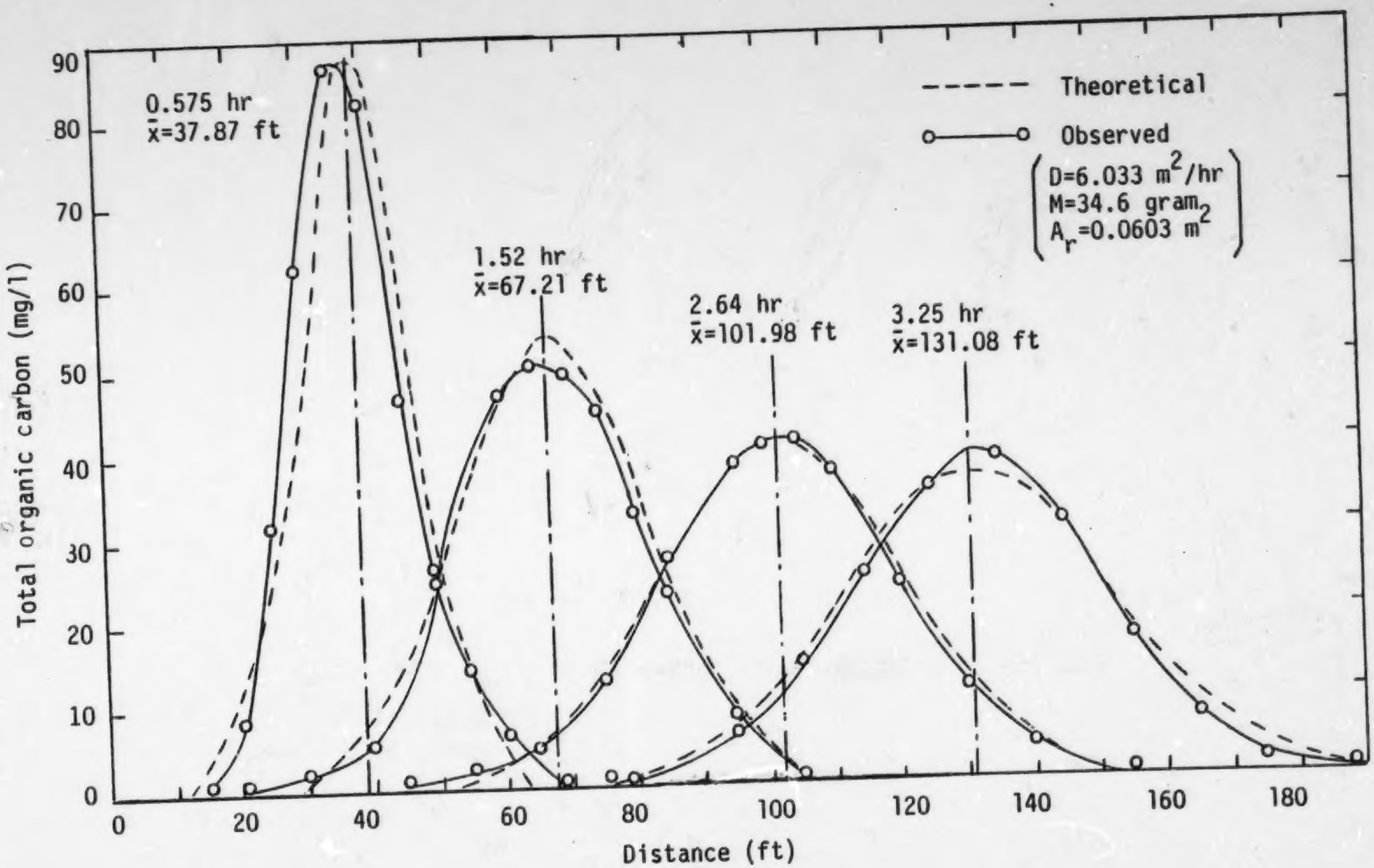

Figure 61. Phenol transportation in the model river (Spill point= 20 ft)

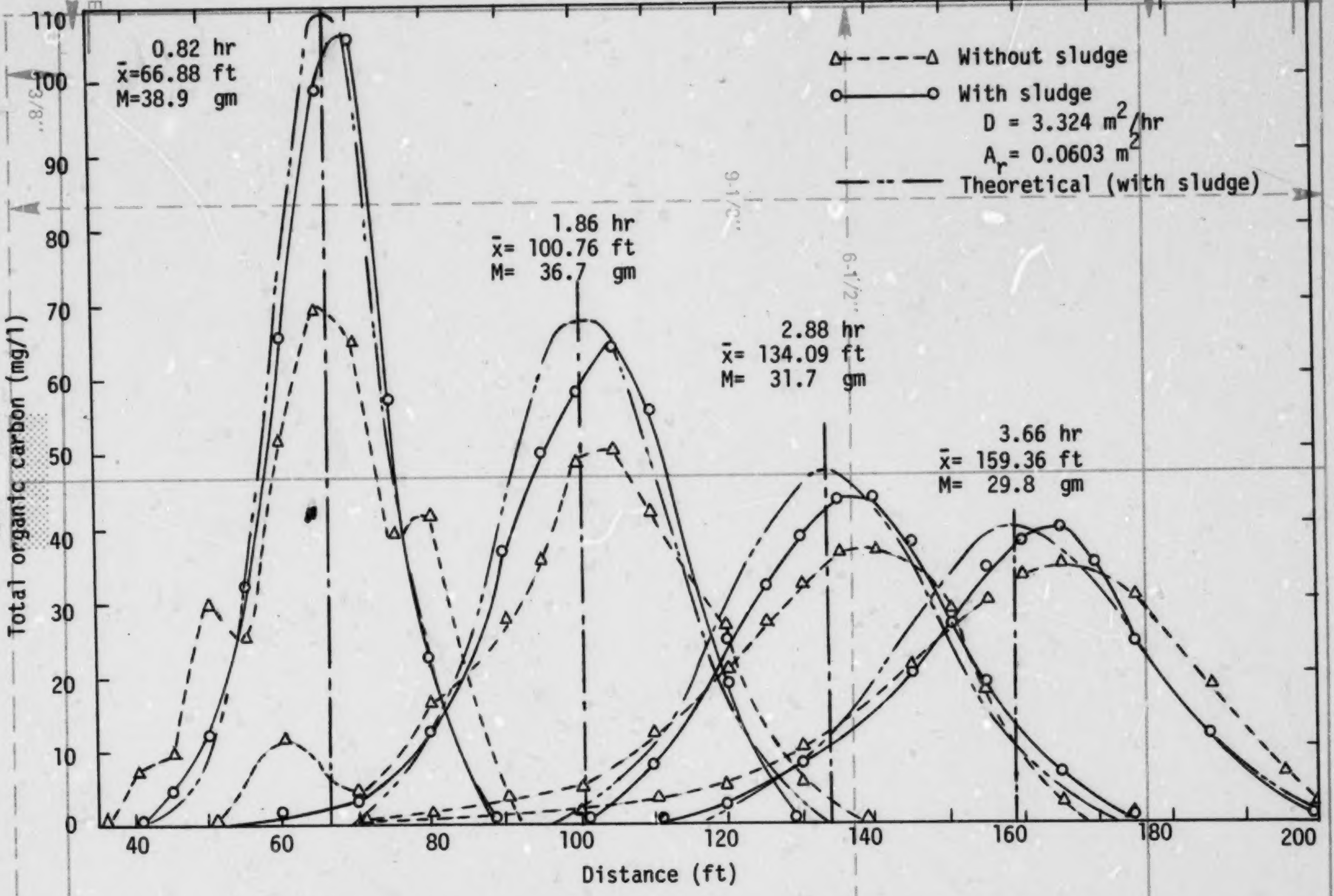

Figure 62. Methanol transportation in the model river (Spill point = 40 ft).

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$$= -\frac{kM_s M_x}{A_r K_s} \frac{1}{2\sqrt{2\pi Dt}} - k_r M_s \; , \tag{95}$$

where M_x is the total mass of VSS applied. At low substrate concentrations, bacterial growth enters the stationary phase (see Fig. 29), so that M_x may be considered constant. Then, the total mass of pollutant at time t is solved by integrating Equation 95. If $t \geq t_a$,

$$\frac{M_s}{M_{so}} = \exp\left\{\frac{kM_x}{\sqrt{2\pi D}\, A_r K_s}\left(\sqrt{t} - \sqrt{t_a}\right) - k_r t\right\} \tag{96}$$

where: M_{so} = total mass spilled,

M_s = total mass at time t (measured from the spillage), and

t_a = sludge application time.

And, if $0 \leq t \leq t_a$,

$$\frac{M_s}{M_{so}} = \exp(-k_r t) \tag{97}$$

A distinct disadvantage of this method can be characterized by the term $\sqrt{t}$ involved in Equation 96. Without enought acclimated activated sluge to compensate for this disadvantage this method may require an unfeasibly long time, as implied by the t within the exponential term.

Unfortunately, most of the sonicated phenol sludge applied over the spills settled down immediately so that there was no measurable amount of suspended organisms left and there was no measurable phenol loss during the transport ($k_r = 0$). More than 60% of the sonicated methanol sludge settled within 45 minutes, and the rest, 39 grams of VSS, remained suspended throughout the test time. There was methanol removal during the transport, but, it was not possible to conclude how much was due to biological degradation (see Figure 63).

Application of Cloth Bags

The usefulness of acclimated activated sludge can be increased by reducing the amount of idle sludge, which is a byproduct of sludge settling and return. If sludge-containing cloth bags are introduced into the reactors, this process is not necessary. Floating cloth bags can also be used for in situ treatment to prevent sludge settling when the mixing intensity is not great enough for sludge suspension. The cloth pores are rapidly clogged with bacterial floc when the bags are filled with concentrated sludge. Through the cloth, liquid exchange is free while the transport of bacterial floc is greatly inhibited.

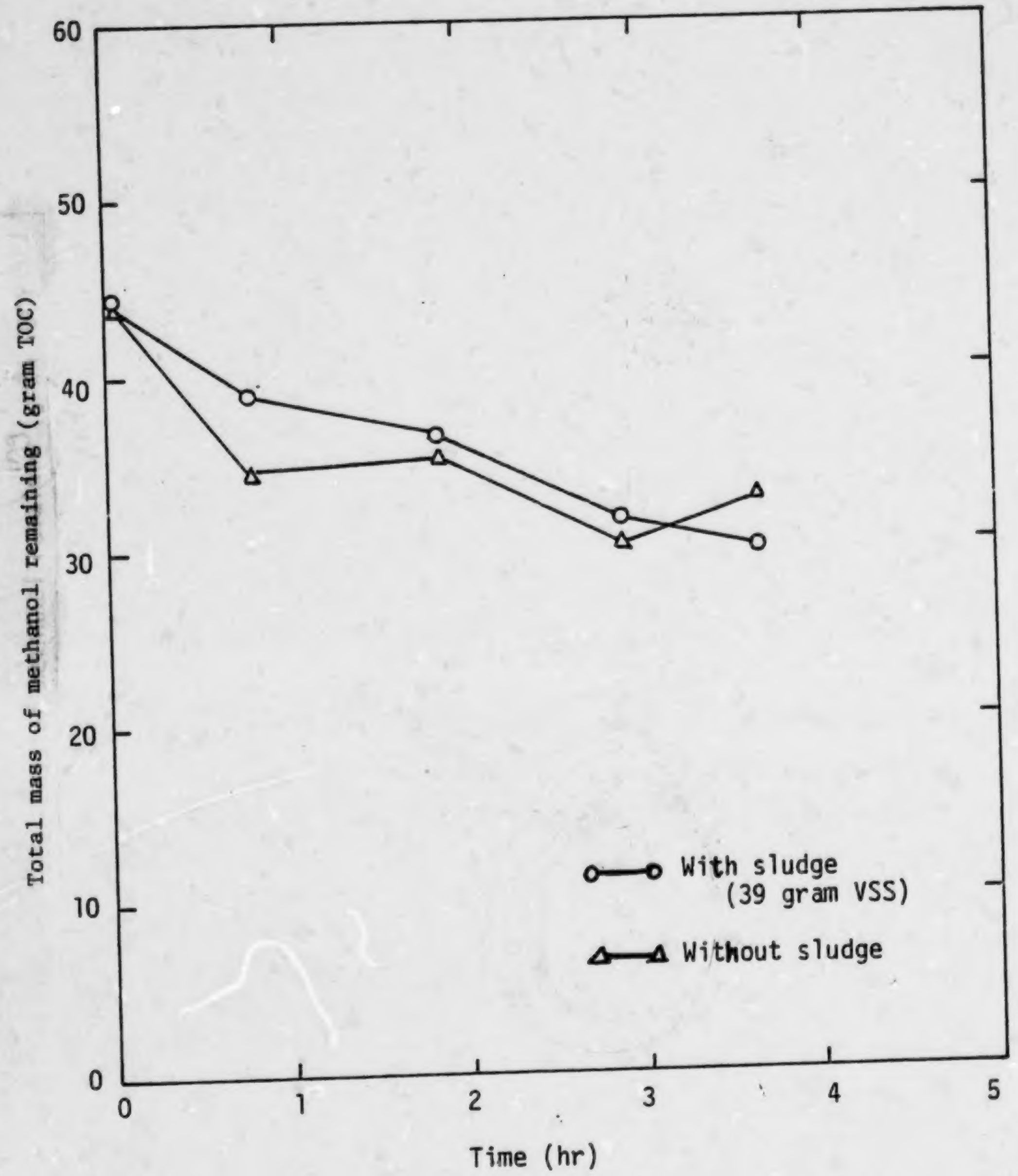

Figure 63. Methanol removal in one-dimensional dispersion system (Temp. = 22°C).

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Material Exchange through Cloth Bags--

The material transport rate into a cloth bag will be proportional to the surface area of the bag and to the concentration gradient. Thus, the following is possible:

$$V_c \frac{dS_c}{dt} = CA_c\,(S-S_c) \qquad (98)$$

where: V_c = volume of cloth bag (L^3),
S = concentration outside the bag (M/L^3),
S_c = concentration inside the bag (M/L^3),
A_c = surface area of the bag (L^2), and
C = material exchange coefficient (L/T).

The material exchange coefficient, C, was estimated using the following methods.

In a confined reactor, the total mass, M, of some substance does not change if the substance is injected inside a cloth bag with inactivated sludge. This mass balance may be described by:

$$V_oS + V_cS_c = M, \qquad (99)$$

where V_o is the volume outside the bag. Thus Equation 97 becomes:

$$\frac{dS}{dt} = \frac{CA_c}{V_oV_c}(M - V_oS - V_cS_c). \qquad (100)$$

Let W denote the difference between the equilibrium concentration and the concentration outside the bag at time t. Then,

$$W = \frac{M}{V} - S, \qquad (101)$$

where,

$$V = V_o + V_c. \qquad (102)$$

Then, Equation 99 becomes:

$$\frac{dW}{dt} = \frac{C\,A}{V_oV_c}\,W \qquad (103)$$

Integration of Equation 103 from time t_1 to t_2 yields:

$$C = \frac{V_oV_c}{A_cV\,(t_2 - t_1)}\ln(W_1/W_2). \qquad (104)$$

In a dispersive stream, the concentration outside a bag is negligible if a substance is injected inside the bag, which is filled with inactivated sludge. Therefore,

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$$\frac{dS_c}{dt} = C \frac{A_c}{V_c} S_c \qquad (105)$$

Integration of Equation 105 from time t_1 to t_2 yields:

$$C = \frac{V_c}{A_c\ (t_2 - t_1)} \ln\ (S_{c1} / S_{c2}) \qquad (106)$$

The estimated material exchange coefficients using Equations 104 and 106 are given in Tables 38 and 39. The coefficients for methanol sludge were twice those for phenol sludge. Even though the concentration of methanol sludge increased from 4,200 mg/l to 6, 760 mg/l (as suspended solids) owing to the sludge settleability, it did not have any perceptible effect on the material exchange rate. In the laminar flow regime, the material exchange coefficient remained at the same level observed in the quiescent condition. The material exchange coefficient showed a linear relationship with velocity in the turbulent flow regime when the flume depth was kept constant (see Figure 64).

Like other phenomena of hydraulic mixing and stirring, the rate of material exchange through cloth bags can also be stated as a function of the mean temporal velocity gradient. Therefore, the following formulation is possible:

$$C = c_1\, G^{c2}, \qquad (107)$$

where:

c_1, c_2 = constants,

G = mean temporal velocity gradient, or mixing intensity

$\left(= \sqrt{\rho g h_f / (\mu t_d)}\right)$ (sec^{-1}),

ρ = density of water (gram/m^3),

g = gravity constant (m/sec^2),

μ = absolute viscosity of water (gram/m/sec),

h_f = head loss in a given stretch (m), and

t_d = detention time in the stretch (sec).

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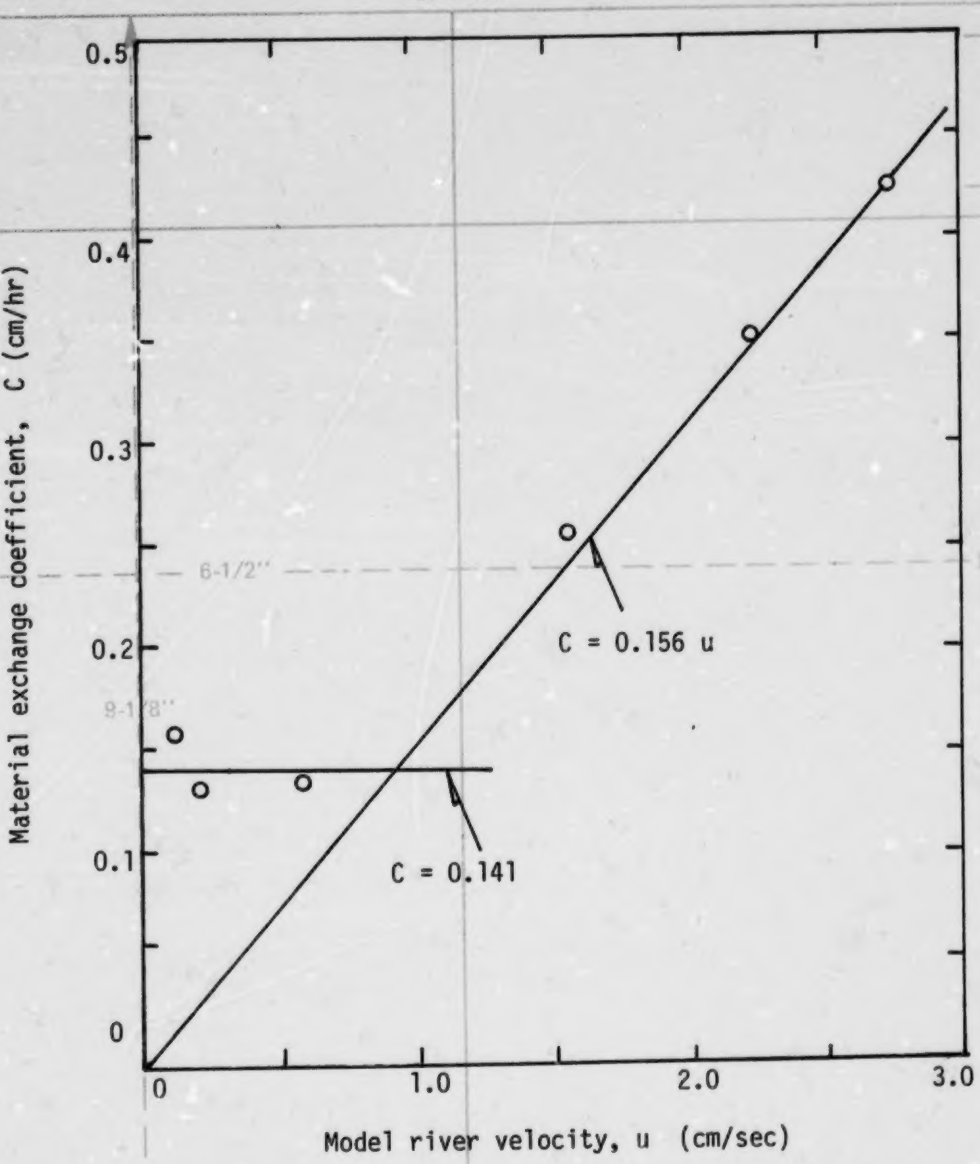

Figure 64. The relationship between stream velocity and material exchange coefficient for methanol sludge.

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TABLE 38. MATERIAL EXCHANGE COEFFICIENT RELATED TO THE TURBULENCE IN CONFINED REACTORS

Sludge	Suspended solids (mg/l)	Volatile S S (mg/l)	Turbulence	Exchange coef., C, (cm/hr)	Number of observations
Phenol	26,130	23,720	Quiescent	0.0710	20
			Turbulent[1]	0.684	20
Methanol	4,200	3,860	Quiescent	0.141	20
			Turbulent[1]	1.350	20

[1] Turbulent conditions were made by aeration through glass tubes with an air flow rate of 1 liter air/min/liter water. The turbulence caused by this air flow rate was just enough for complete suspension of phenol-acclimated sludge without cloth bags.

TABLE 39. MATERIAL EXCHANGE COEFFICIENT RELATED TO THE STREAM VELOCITY IN THE MODEL RIVER (METHANOL SLUDGE[1])

Velocity (cm/sec)	Reynolds No.	Exchange coef. (cm/hr)	Flow regime[2]	Number of observations
0.106	440	0.155	Laminar	3
0.191	800	0.131	Laminar	3
0.575	2,400	0.133		3
1.551	6,500	0.256	Turbulent	3
2.250	9,400	0.349	Turbulent	4
2.731	11,400	0.425	Turbulent	4

[1] SS = 6,760 mg/l, VSS = 6,270 mg/l, temperature = 28 °C, hydraulic radius = 0.124 m.

[2] Flow regimes were visually observed using a Rhodamine B dye solution.

If Manning's formula is employed to estimate the head loss and the detention time, c_2 should be 2/3 in order to describe the linear relationship between the exchange coefficient and the velocity in the turbulent regime. Manning's formula is expressed as:

$$u = \frac{1}{n} I^{1/2} R^{2/3}, \tag{108}$$

where: u = mean stream velocity (m/sec),
n = coefficient of roughness,
I = energy gradient, and
R = hydraulic radius in m.

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Therefore,

$$G = \left(\frac{\rho g n^2 \mu^3}{\mu R^{4/3}}\right)^{1/2} \tag{109}$$

If the coefficient of roughness, n, of 0.025 is selected for a test channel with moderate weeds (Davis, 1952), the following is proposed for prediction of the material exchange coefficient in the turbulent flow:

$$C = 0.159\ G^{2/3} \text{ (for phenol sludge) and} \tag{110}$$

$$C = 0.317\ G^{2/3} \text{ (for methanol sludge),} \tag{111}$$

where C has the units cm/hr, and G, sec^{-1}.

Reaeration in streams is also thought to be a result of hydraulic mixing. Camp and Meserve (1974) brought together a number of observations on large rivers and on an experimental channel and interpreted reaeration as a function of the mean temporal velocity gradient. Fair et al, (1968) approximated the Camp and Meserve's analysis as:

$$k_a H = 29\ G^{2/3}$$

where k_a is the reaeration coefficient (day^{-1}), H is the hydraulic depth of the stream (ft), and G is the mean temporal velocity gradient (sec^{-1}). Since $k_a H$ is equivalent to C in this study, Equations 110 and 111 agree with their analysis.

Efficiency of Cloth Bag --

The mass transport rate into a cloth bag should equal the mass removal rate by the organisms inside the bag. Therefore,

$$V_c \frac{k X_c S_c}{K_s + S_c} = CA(S - S_c), \tag{112}$$

where X_c = VSS concentration inside the bag (mg/l).

Thus, the substrate concentration inside the bag (S_c) is solved to be:

$$S_c = \frac{S - \frac{V_c}{A_c}\frac{k}{C}X_c - K_s + \sqrt{\left(S - \frac{V_c}{A_c}\frac{k}{C}X_c - K_s\right)^2 + 4K_s S}}{2}. \tag{113}$$

Let the cloth bag efficiency, E, denote the ratio of substrate removal rate inside the bag to the removal rate without the bag, or:

$$E = \left(\frac{kX_c S_c}{K_s + S_c}\right) \Big/ \left(\frac{kX_c S}{K_s + S}\right) = \frac{S_c (K_s + S)}{S (K_s + S_c)}. \tag{114}$$

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Thus, E is a function of the substrate concentration (S), the cloth bag shape factor (V_c/A_c), the substrate removal rate coefficient (k), the material exchange coefficient (C), and the biomass concentration inside the bag (X_c). Among the above factors, X_c is the least important factor in the range of 15,000 mg/l to 25,000 mg/l (as VSS) for phenol sludge and 4,000 mg/l to 6,000 mg/l (as VSS) for methanol sludge. The cloth bag efficiency diagrams for phenol and methanol sludge are presented in Figures 65-1 to 65-9 and 66-1 to 66-9 for various values of k, C, and S. The main purpose of these diagrams is for the proper design of cloth bags under various circumstances. Use of the diagrams is as follows:

First, find a diagram for a given or desired set of k and C values. Then, from the diagram, find the cloth bag efficiency corresponding to the cloth bag shape factor, V_c/A_c, and the substance concentration range (between the spill concentration and the desired concentration after treatment).

The cloth bag efficiency decreases as the cloth bag shape factor (V_c/A_c) increases, as the substance concentration decreases, as C decreases, and as k increases.

Application of Cloth Bags in Confined Reactors--

When the sludge-containing cloth bags are used in a confined, batch reactor, the total mass removal rate by organisms is expressed as:

$$V\frac{dS}{dt} = NV_c\,\frac{EkX_cS}{K_s+S}, \qquad (115)$$

where N is the number of cloth bags. Therefore,

$$\frac{dS}{dt} = \frac{(Ek)XS}{K_s+S} \qquad (116)$$

where:

$$X = \frac{NV_cX_c}{V}. \qquad (117)$$

Because there is no change in the bacterial growth kinetics, the only difference between two batch treatment systems with and without cloth bags is that Ek, instead of k, should be considered as the substrate removal rate coefficient in the cloth bag systems. If E remains almost constant throughout the reaction period (between the spill concentration and the desired effluent concentration), the system can be designed using the methods described in the section entitled Design of Batch Treatment System, with a substitution of Ek for k. If E changes with substrate concentration, the cloth bag efficiency should be estimated at each station using Eqs. 104 and 105, before entering the iteration process of Eqs. 72 and 73.

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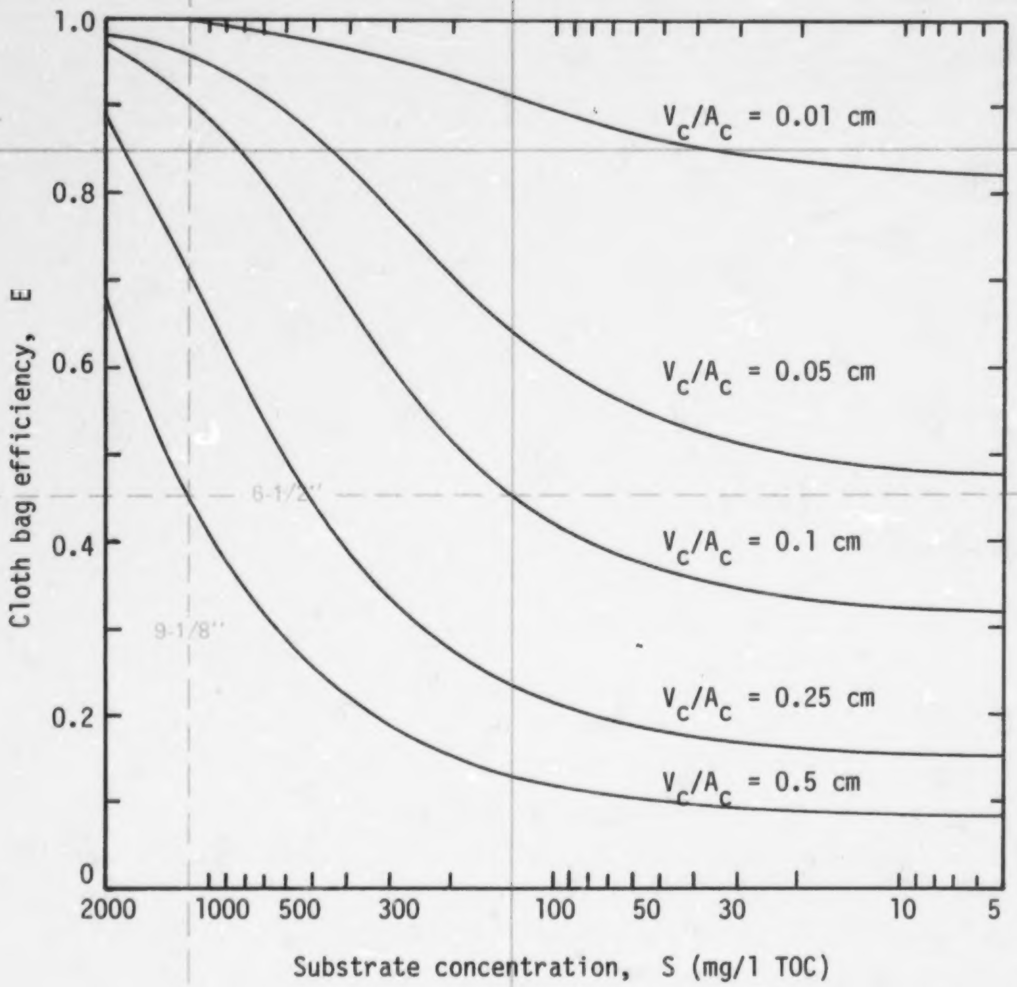

Figure 65-1. Efficiency of cloth bag filled with phenol sludge ($k = 0.01892\ hr^{-1}$, $C = 0.07$ cm/hr, and $X_c = 20,000$ mg/l).

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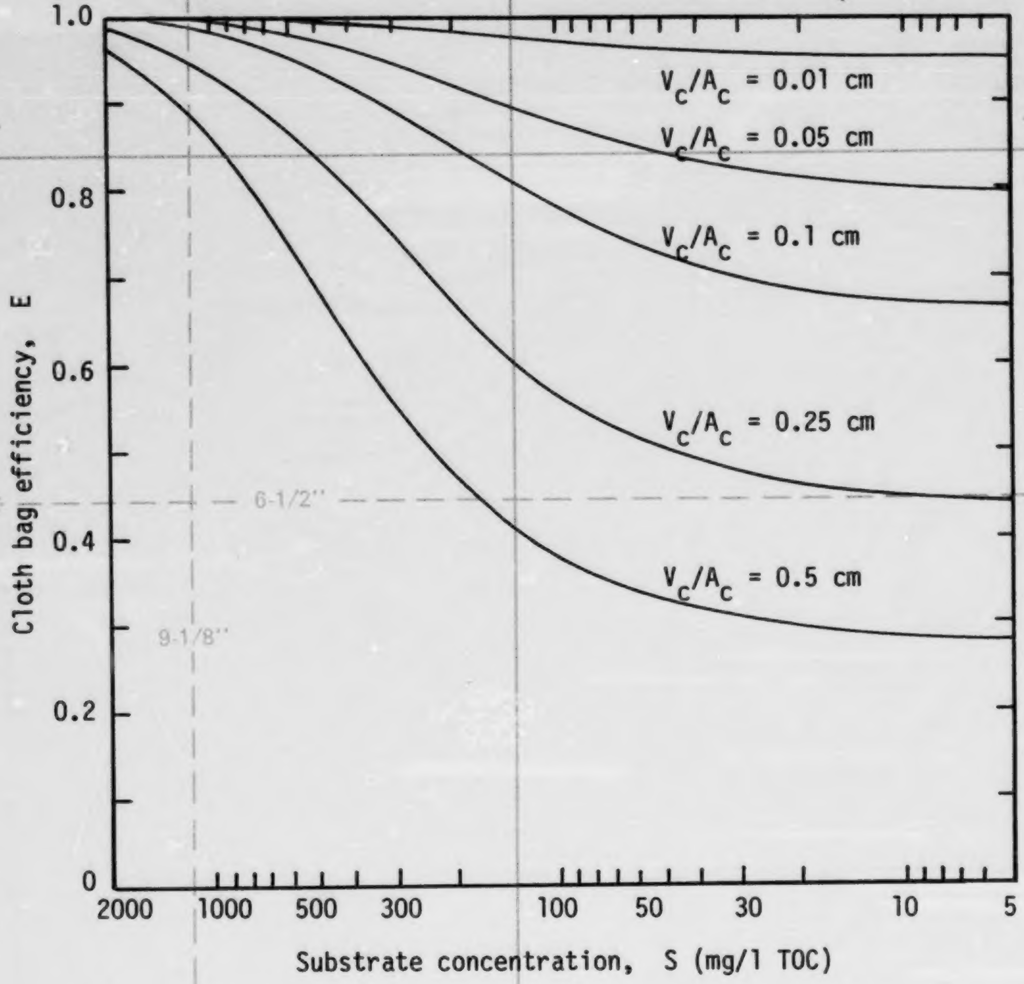

Figure 65-2. Efficiency of cloth bag filled with phenol sludge ($k = 0.01892\ hr^{-1}$, $C = 0.3$ cm/hr, and $X_c = 20{,}000$ mg/l).

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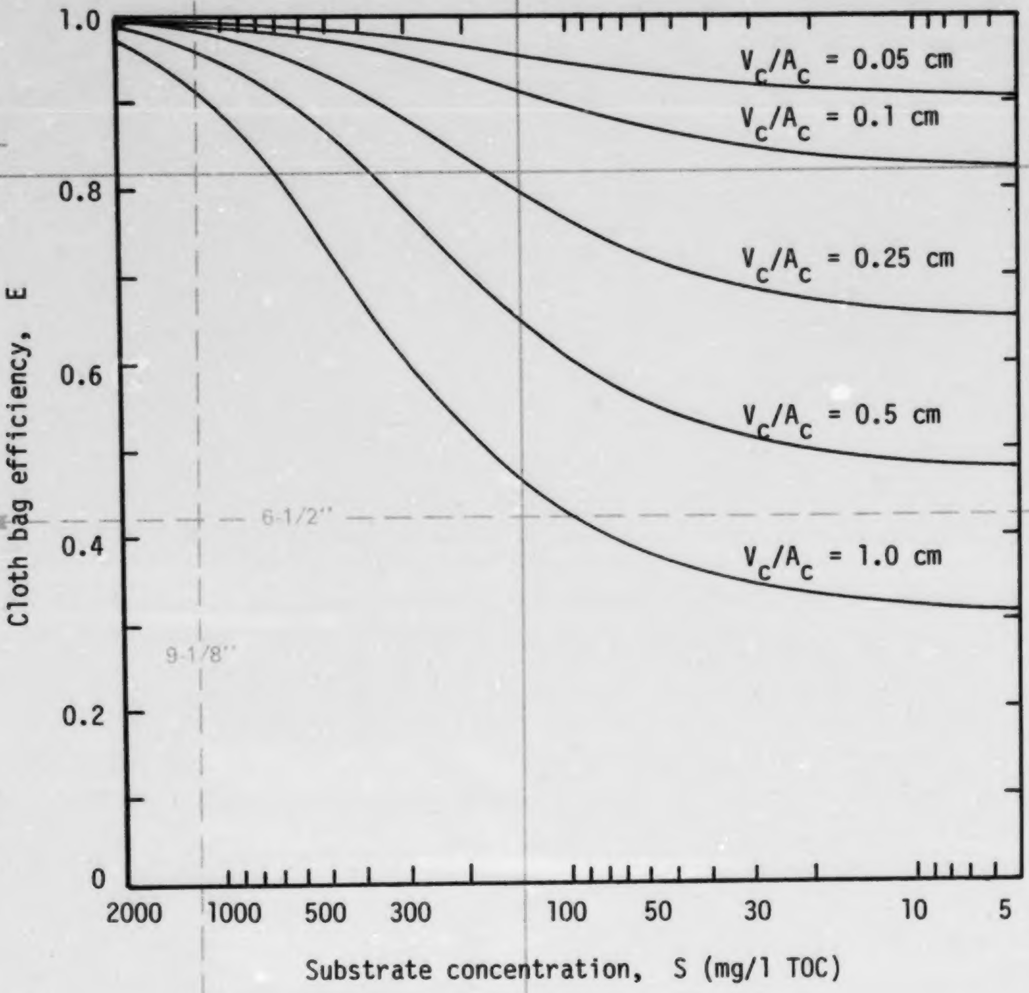

Figure 65-3. Efficiency of cloth bag filled with phenol sludge ($k = 0.01892\ hr^{-1}$, $C = 0.7$ cm/hr, and $X_c = 20{,}000$ mg/l).

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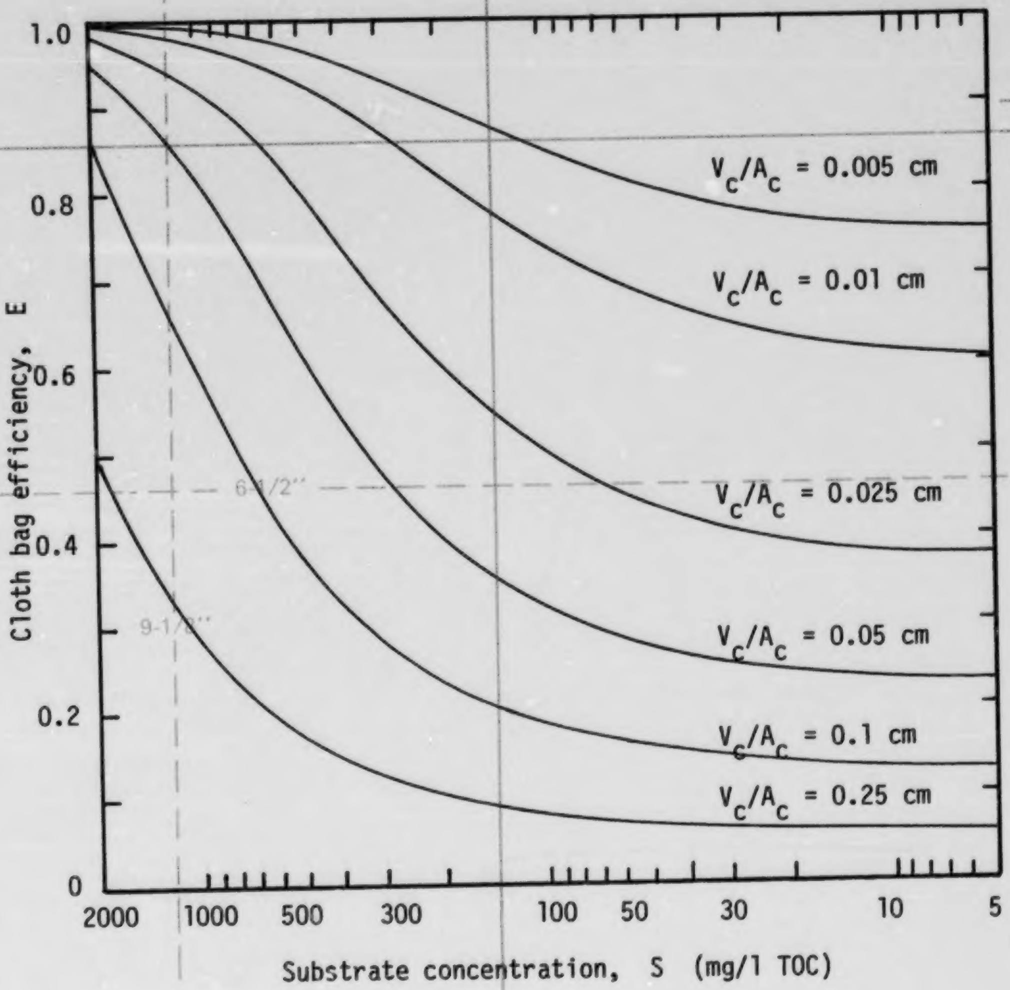

Figure 65-4. Efficiency of cloth bag filled with phenol sludge ($k = 0.05674\ \text{hr}^{-1}$, $C = 0.07$ cm/hr, and $X_c = 20,000$ mg/l).

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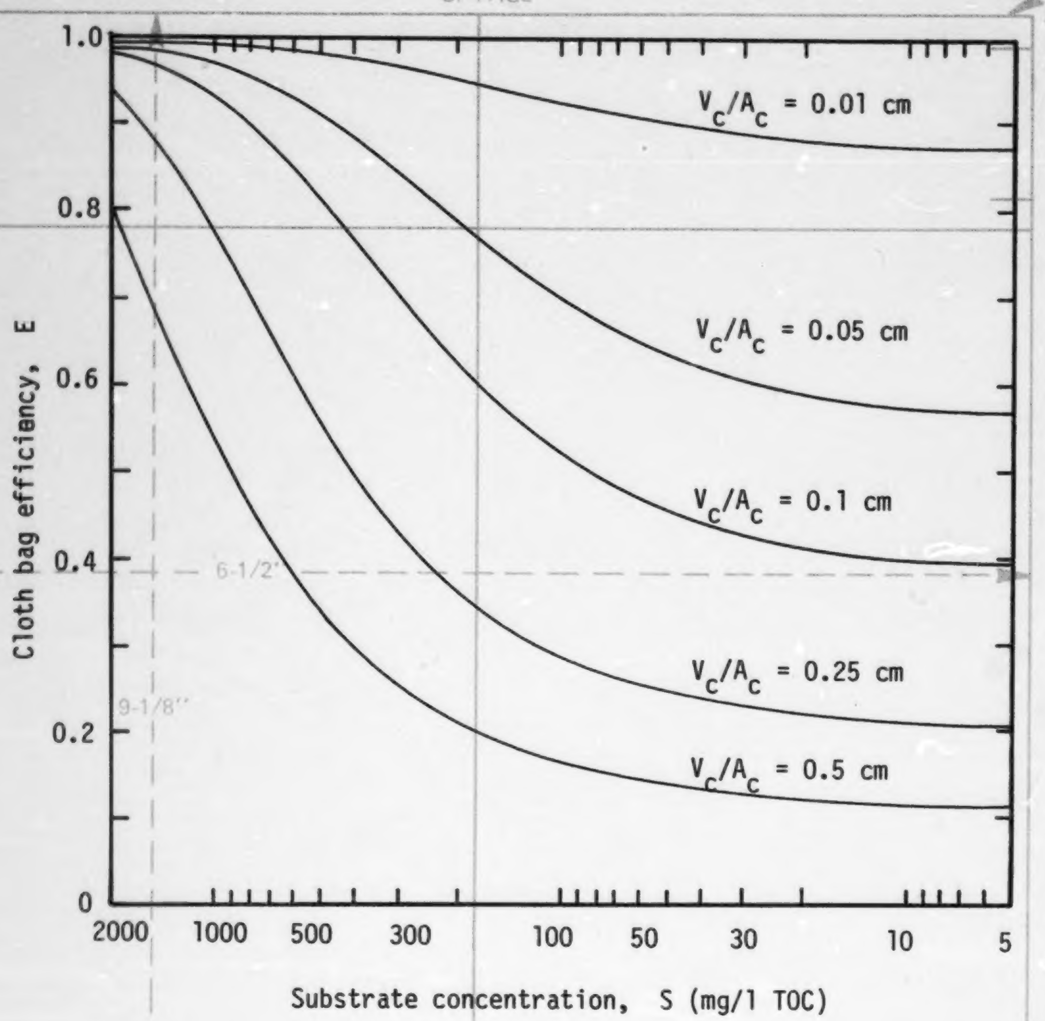

Figure 65-5. Efficiency of cloth bag filled with phenol sludge ($k = 0.05674\ hr^{-1}$, $C = 0.3$ cm/hr, and $X_c = 20{,}000$ mg/l).

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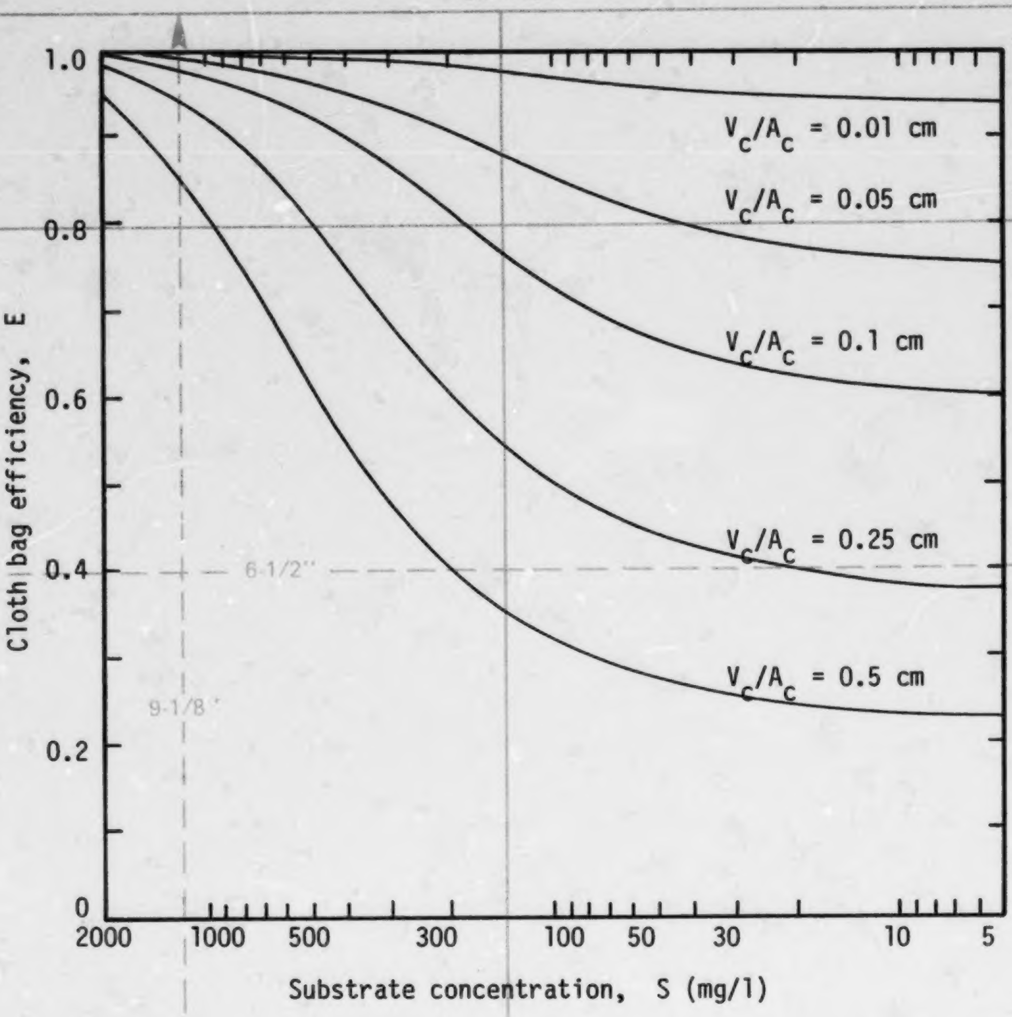

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Figure 65-6. Efficiency of cloth bag filled with phenol sludge ($k = 0.05674\ hr^{-1}$, $C = 0.7$ cm/hr , and $X_c = 20{,}000$ mg/l).

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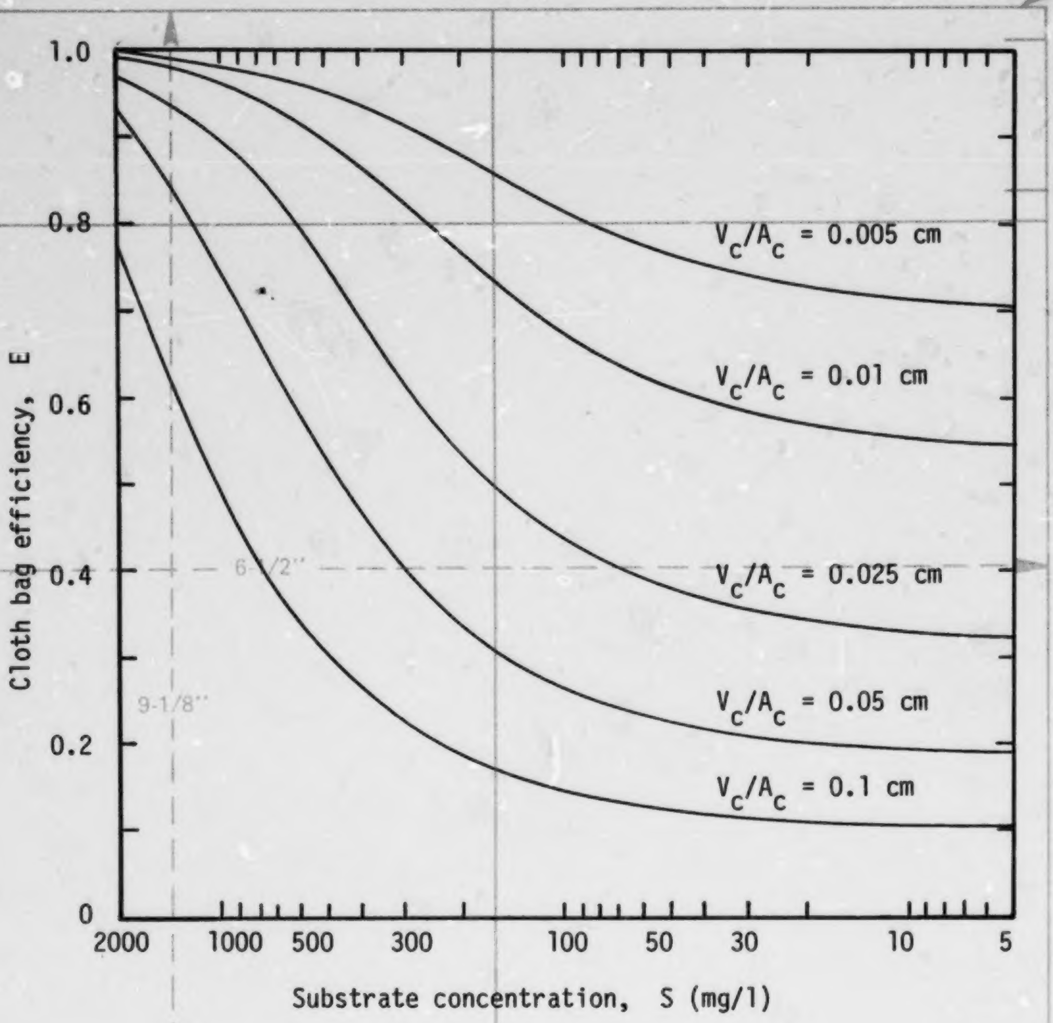

Figure 65-7. Efficiency of cloth bag filled with phenol sludge ($k = 0.07239\ hr^{-1}$, $C = 0.07$ cm/hr, and X_c = 20,000 mg/l).

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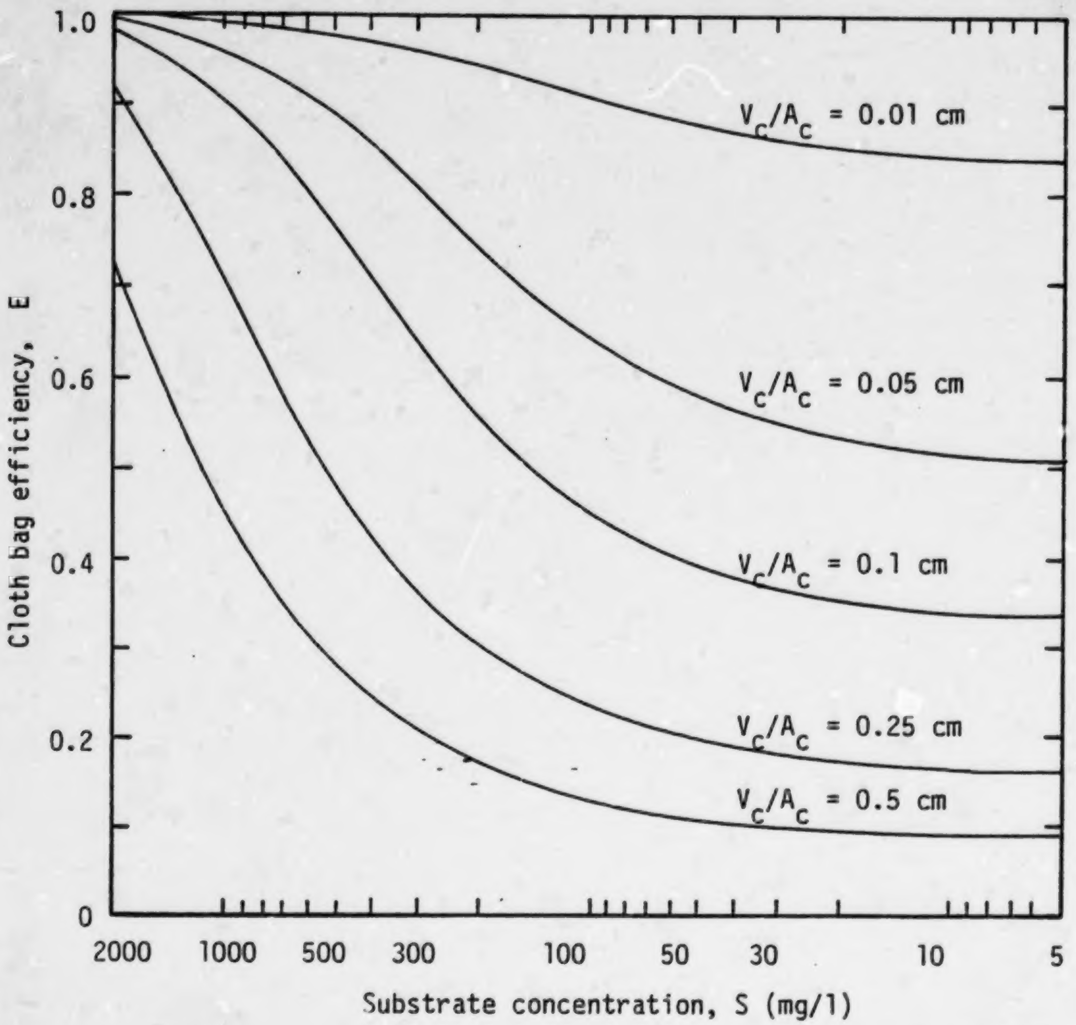

Figure 65-8 Efficiency of cloth bag filled with phenol sludge ($k = 0.07239\ hr^{-1}$, $C = 0.3$ cm/hr , and $X_c = 20{,}000$ mg/l).

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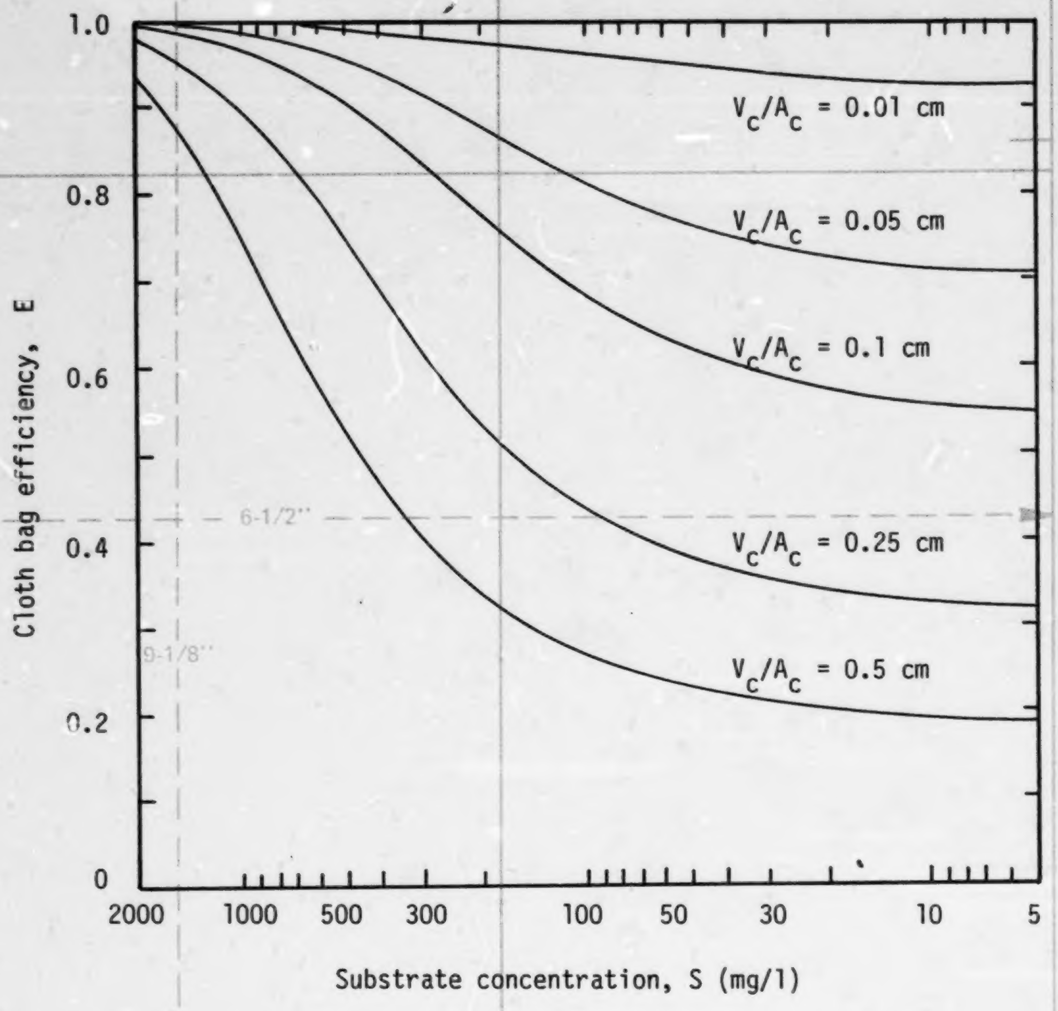

Figure 65-9. Efficiency of cloth bag filled with phenol sludge ($k = 0.07239\ hr^{-1}$, $C = 0.7$ cm/hr, and $X_c = 20,000$ mg/l).

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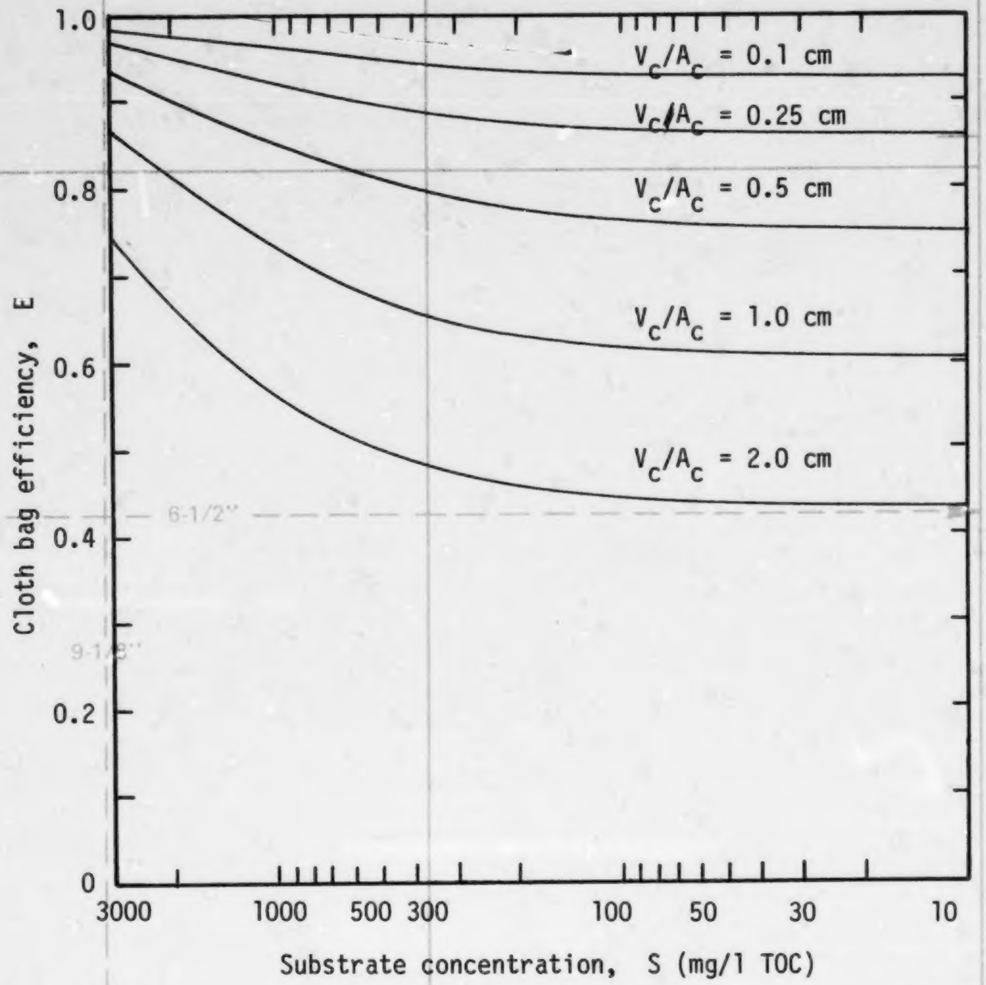

Figure 66-1. Efficiency of cloth bag filled with methanol sludge ($k = 0.04407\ hr^{-1}$, $C = 0.14$ cm/hr, and $X_c = 5{,}000$ mg/l).

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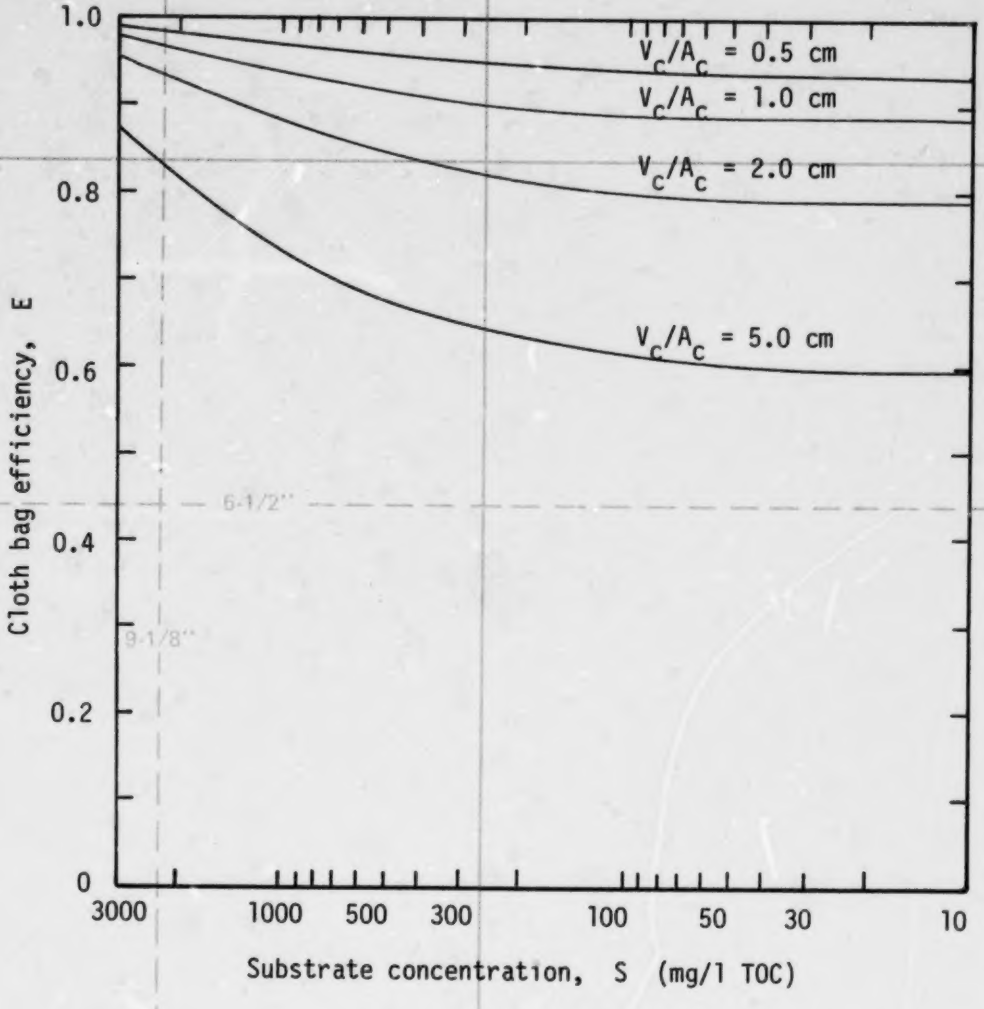

Figure 66-2. Efficiency of cloth bag filled with methanol sludge ($k = 0.04407\ hr^{-1}$, $C = 0.7$ cm/hr , and $X_c = 5,000$ mg/l).

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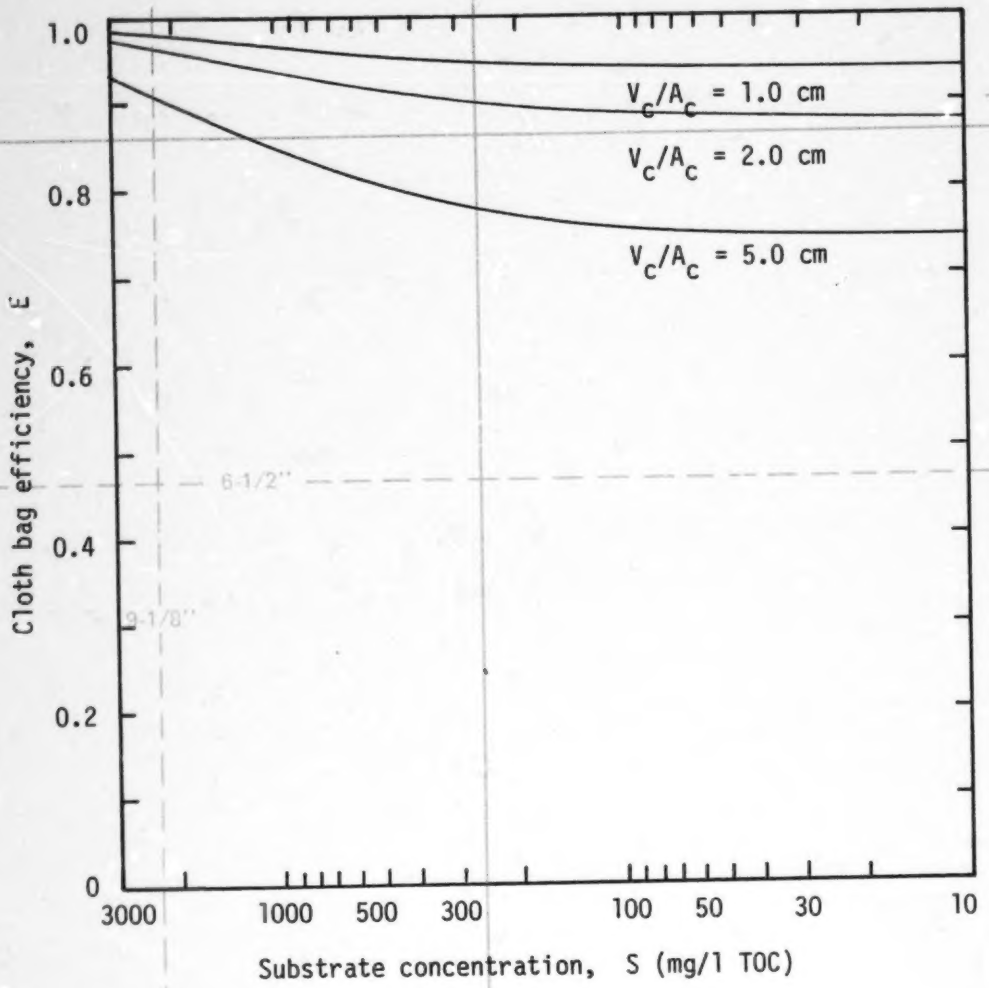

Figure 66-3. Efficiency of cloth bag filled with methanol sludge ($k = 0.04407\ hr^{-1}$, $C = 1.35$ cm/hr, and $X_c = 5{,}000$ mg/l).

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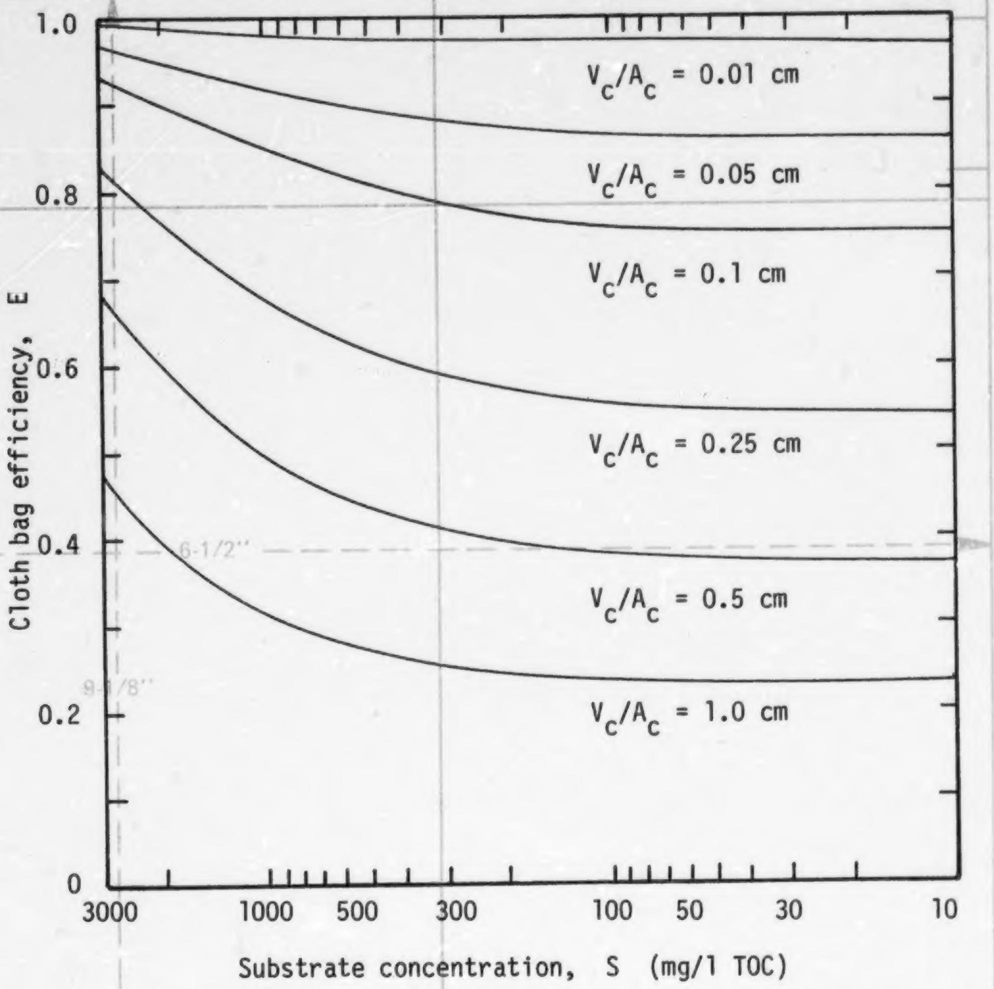

Figure 66-4. Efficiency of cloth bag filled with methanol sludge ($k = 0.2256\ hr^{-1}$, $C = 0.14$ cm/hr, and $X_c = 5{,}000$ mg/l).

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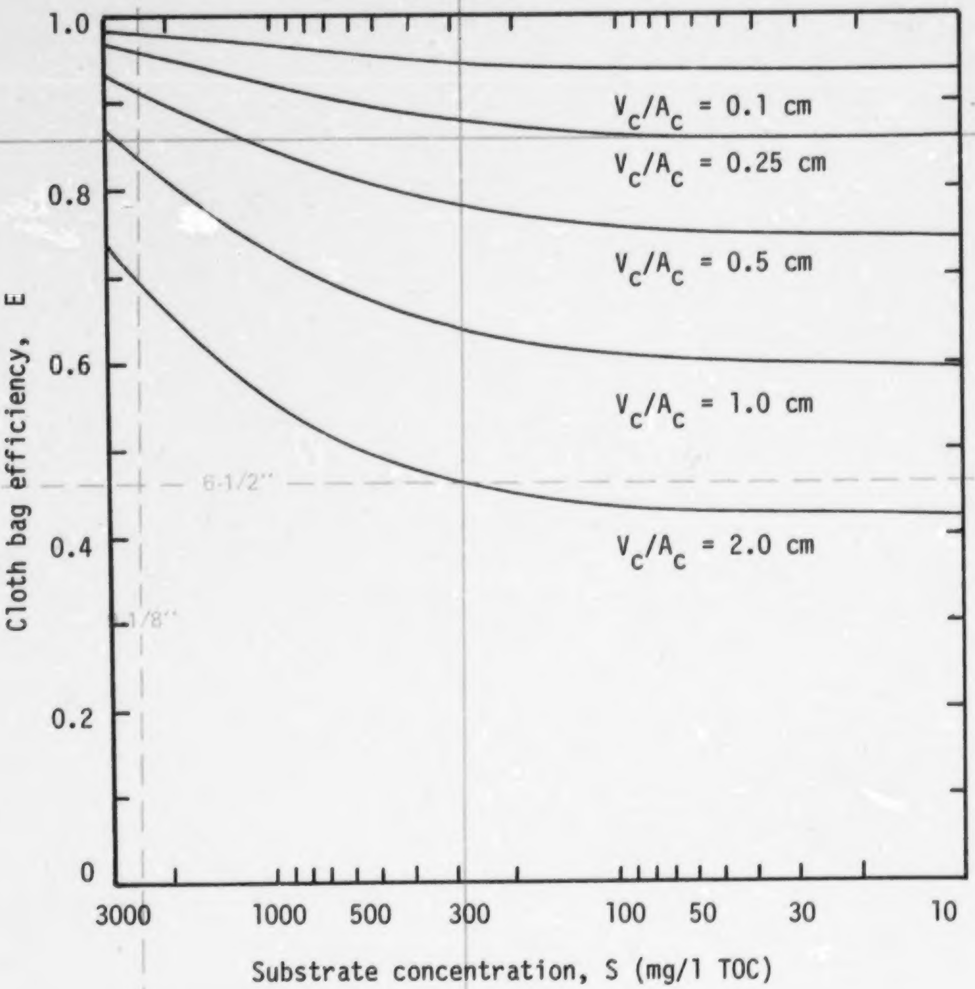

Figure 66-5. Efficiency of cloth bag filled with methanol sludge ($k = 0.2256\ hr^{-1}$, $C = 0.7$ cm/hr, and $X_c = 5,000$ mg/l).

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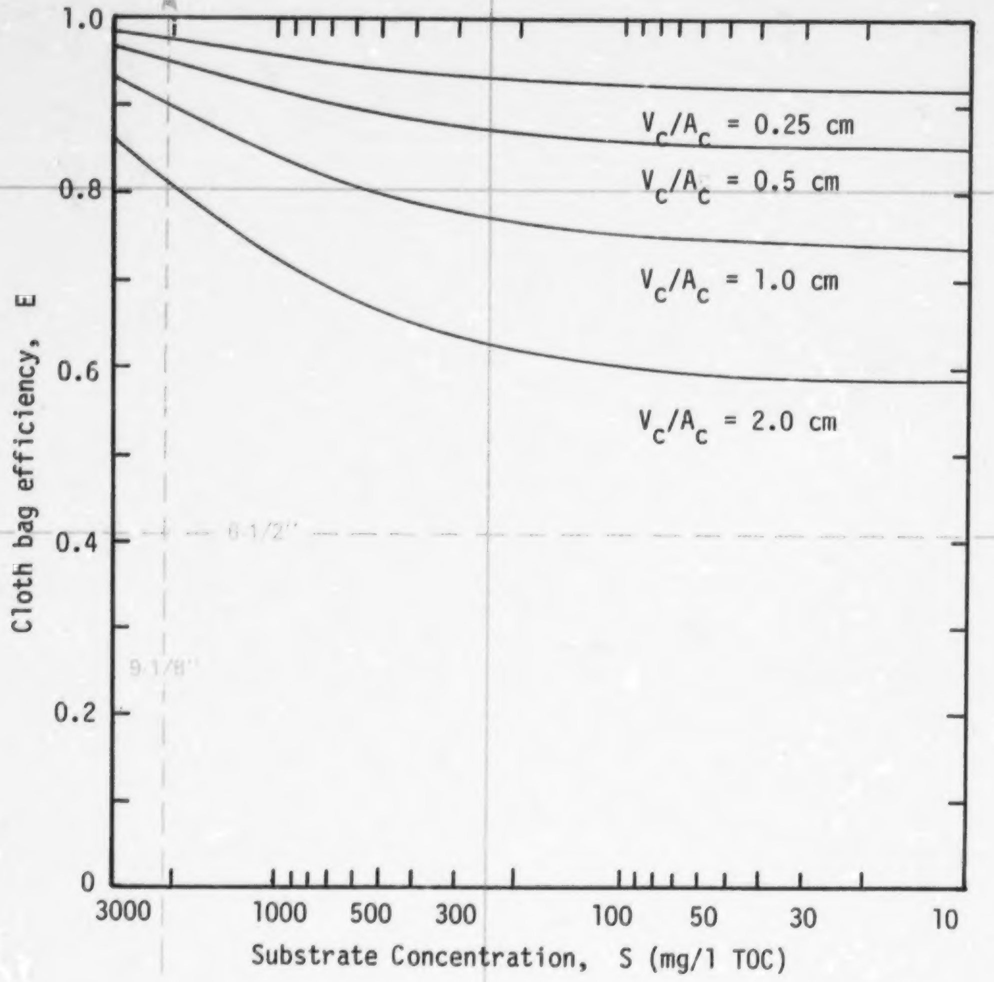

Figure 66-6. Efficiency of cloth bag filled with methanol sludge ($k = 0.2256\ hr^{-1}$, $C = 1.35$ cm/hr, and $X_c = 5,000$ mg/l).

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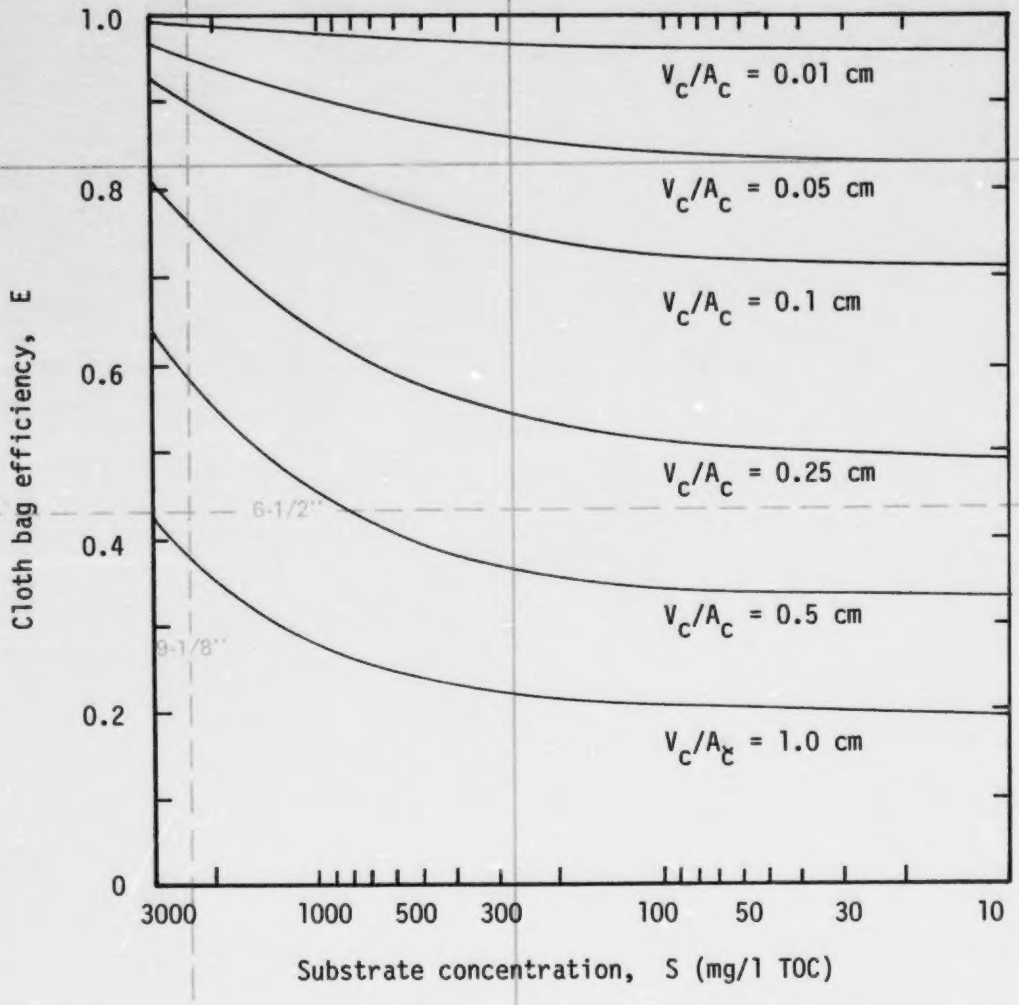

Figure 66-7. Efficiency of cloth bag filled with methanol sludge ($k = 0.2656\ hr^{-1}$, $C = 0.14$ cm/hr, and $X_c = 5,000$ mg/l).

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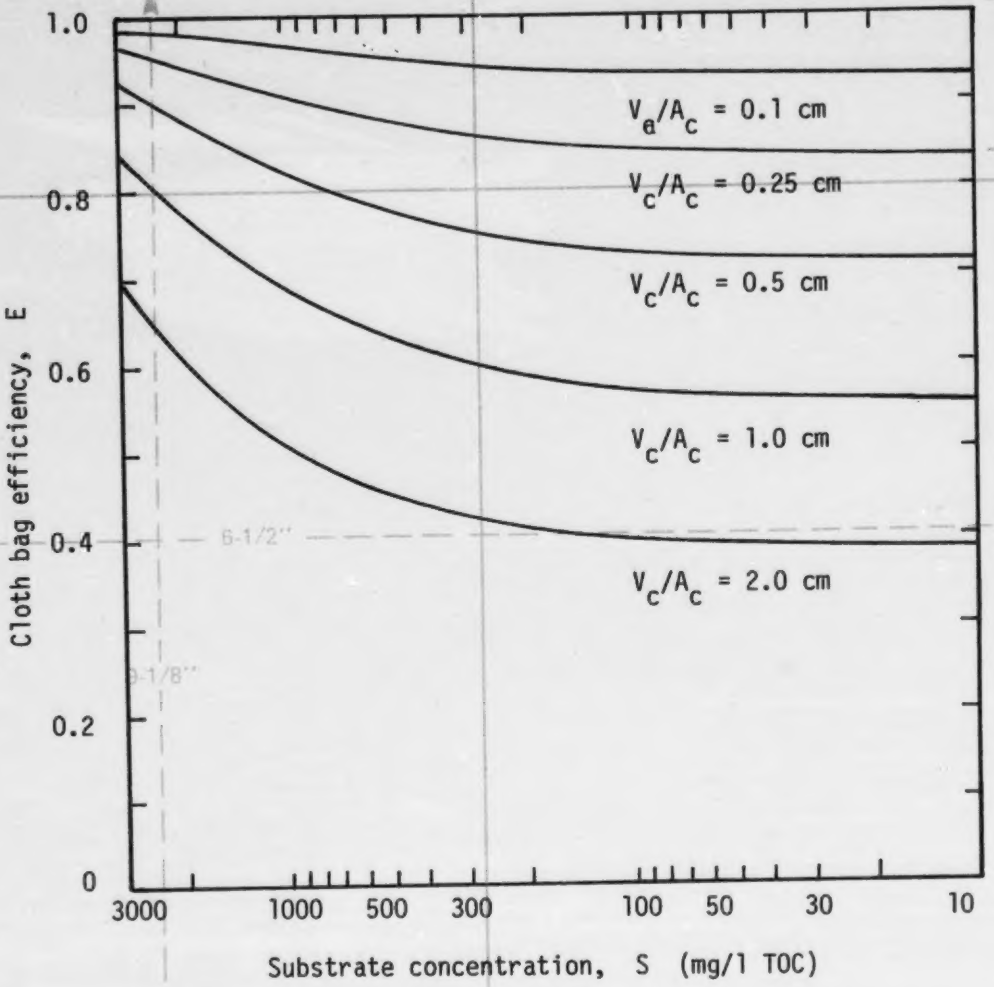

Figure 66-8. Efficiency of cloth bag filled with methanol sludge ($k = 0.2656$ hr^{-1}, $C = 0.7$ cm/hr, and $X_c = 5{,}000$ mg/l).

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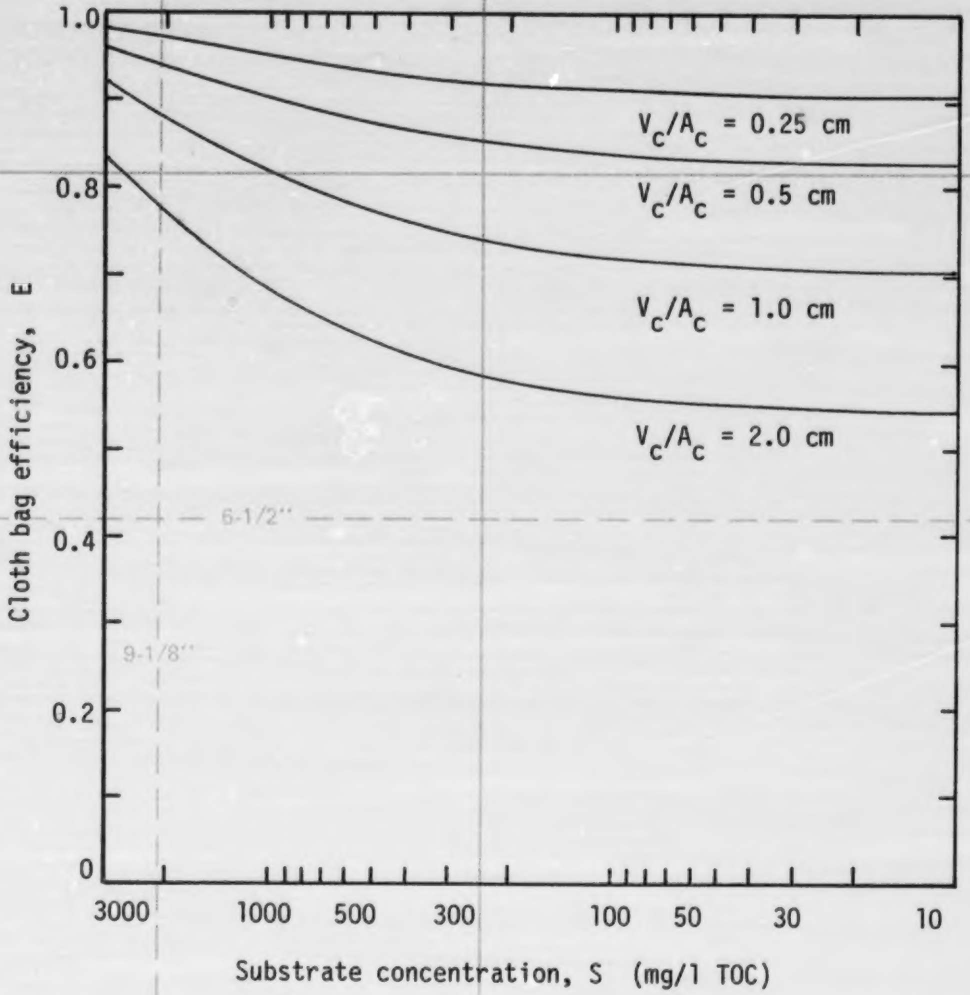

Figure 66-9. Efficiency of cloth bag filled with methanol sludge ($k = 0.2656 \text{ hr}^{-1}$, $C = 1.35$ cm/hr , and $X_c = 5{,}000$ mg/l).

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The theoretically computed substrate removal patterns using the latter method are compared to the observed ones in Fig. 67. The tests were conducted at 24 °C with all nutrients and pH buffer provided, distilled water as a dilution water, and methanol as the substrate. The stripping rate coefficient of 0.0181 hr^{-1} was estimated from a separate reactor with the same conditions except for the acclimated activated sludge. All other necessary kinetic coefficients were estimated from diagrams and equations defined in this study. Detailed experimental conditions and necessary kinetic information are tabulated in Table 40.

TABLE 40. EXPERIMENTAL CONDITIONS AND KINETIC INFORMATION FOR THE METHANOL REMOVAL BATCH TESTS USING CLOTH BAGS

Parameter	Condition	Source
Temperature	24.0 °C	
pH	6.9	
Dilution water	Distilled water	
Nutrients (Nitrogen, Phosphorus and minerals)	Provided	
Cloth bag shape factor (V_c/A_c)	0.672 cm (#1), 0.648 cm (#2), 0.695 cm (#3)	
K_s	2,330 mg/l	
a	1.25	
k	0.28 hr^{-1}	Figs. 24, 27 and Eq. 3
k_d	0.00513 hr^{-1}	Eq. 71
C	1.35 cm/hr	Table 38
Initial X (mg/l)	6,780 (#1), 7,040 (#2), 6,400 (#3)	
(mg/l) Initial X	380	

A total of 1.14 grams of VSS were applied initially in 3-liter reactors (X = 380 mg/l). After 40 hours of aeration, total VSS was measured to be about 2.25 grams and the VSS that had escaped from cloth bags was less than 90 mg (30 mg/l). Thus, less than 10% of the initial biomass applied escaped from the bags and there was a net increase of biomass inside the bags.

The aeration time required to obtain a given substrate removal was observed to be about 5% less than that computed. The organisms that escaped from bags are free from the cloth bag barrier (E = 1.0) and dilute biomass concentrations outside the bags produced improved k values, as pointed out previously.

For detailed computation results for the cloth bag efficiency, biomass growth, and aeration time required, see Appendix B.

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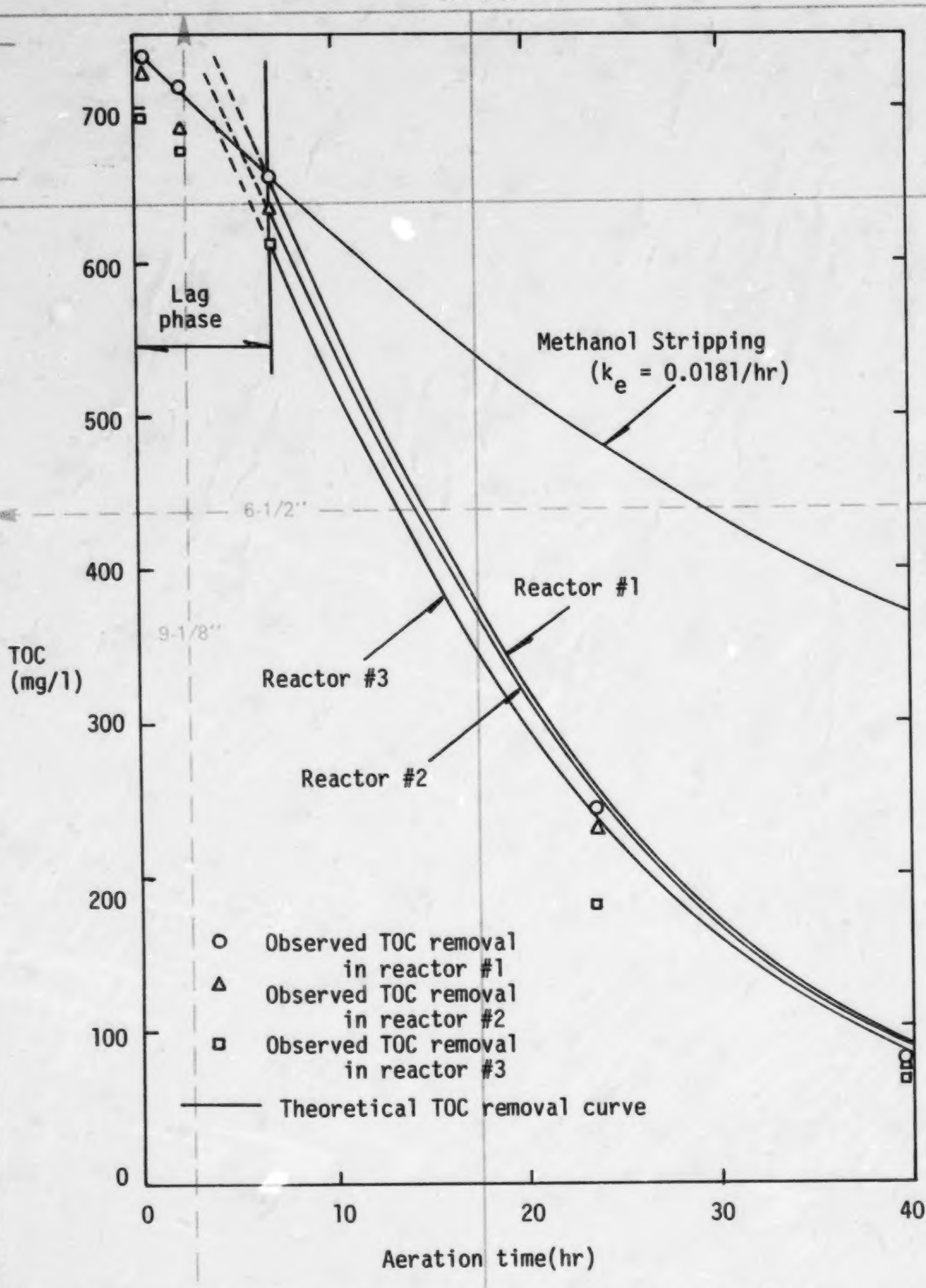

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Figure 67. Methanol removal using cloth bags in batch reactors.

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Application of Cloth Bags in One-Dimensional Dispersion

System--

When N number of sludge-containing cloth bags are deployed over the spills according to the pollutants dispersion model, the distribution function of cloth bags can be given by the following equation, assuming that the dispersion coefficient of cloth bags is the same as the pollutant dispersion coefficient:

$$f_c = \frac{N}{\sqrt{4\pi Dt}} \exp\left\{-\frac{(x-ut)^2}{4Dt}\right\}^*, \qquad (118)$$

where the dimension of f_c is l/L. Then, the total mass removal rate can be expressed as:

$$\frac{dM_s}{dt} = -\int_{-\infty}^{\infty} \frac{E k V_c f_c X_c S}{K_s + S}\, dx - \int_{-\infty}^{\infty} A_r k_r S\, dx. \qquad (119)$$

When the peak spill concentration is considerably smaller than K_s, the cloth bag efficiency becomes almost constant with substrate concentration (see Figures 65 and 66). Then, the total mass of the pollutant at time t is obtained by integrating Equation 119 from the cloth bag application time, t_a, to t, or:

$$\frac{M_s}{M_{s0}} = \exp\left\{-\frac{E k M_x}{\sqrt{2\pi D}\, A_r K_s} \left(\sqrt{t} - \sqrt{t_a}\right) - k_r t\right\}, \qquad (120)$$

where M_x = total biomass applied (= N $V_c X_c$).

Oxygen Requirement Considerations in the Application of Cloth Bags

The oxygen transport rate into a cloth bag should balance the oxygen consumption rate by the organisms inside the bag. When the dissolved oxygen (D.O.) level inside the bag is designed to be zero, the following equation is possible:

$$V_c\left(a' \frac{E k X_c S}{K_s + S} + b' X_c\right) = C A_c O, \qquad (121)$$

where O = DO level outside bags (mg/l).

Thus, the required DO level outside the cloth bags is solved to be:

$$O = \frac{1}{C} \frac{V_c}{A_c} \left(\frac{a' E k X_c S}{K_s + S} + b' X_c\right). \qquad (121')$$

The cloth bag shape factor, V_c/A_c, or the amount of sludge inside the bag, X_c, needs to be designed so that the required DO level does not exceed the saturated level to avoid anaerobic conditions inside the bag.

---

* The dispersion coefficient for cloth bags was assumed to be the same as that for pollutants because the difference does not affect the pollutant removal rate significantly. See Appendix C for more details.

In a confined reactor, the oxygen supply rate into the system should equal the oxygen consumption rate by organisms.

Thus,

$$V k_a(O_s - O) = NCA_cO, \qquad (122)$$

where: V = volume of reactor (L^3),

k_a = aeration rate coefficient (1/T),

O_s = saturated DO level (M/L^3), and

N = number of cloth bags.

Therefore, the aeration devices should be able to provide oxygen at the aeration rate coefficient, k_a, or

$$k_a = \frac{NA_c}{V} C \frac{O}{O_s - O} \qquad (122')$$

In a stream, the oxygen supply and consumption rate balance is expressed as:

$$A_r k_a(0_s - 0) = f_c CA_c 0. \qquad (123)$$

The number of cloth bags per unit length of streams (f_c) is restricted by the reaeration abilities of streams (k_a), or:

$$f_c = \frac{A_r}{A_c} \frac{k_a}{C} \frac{O_s - O}{O} \qquad (123')$$

Therefore cloth bags applied in excess of this number will cause an aerobic conditions within the bags.

The above gas transfer mechanisms deal with mechanical transport only. If oxygen is supplied at the cloth site by means of air diffusers, rather than by mechanical mixing, the above restrictions will be greatly alleviated. In addition to the oxygen supplied from the surrounding water by mechanical mixing, the cloth bags will receive oxygen through that portion of the bag that is in contact with air. This mechanism has not been studied.

Application of Material Confining Barriers

Containment of a spill in a concentrated form provides better treatment efficiency and, in addition, reduces damage to the total environment. Brown (1972) reported that an inflatable plastic barrier was highly effective in confining phenol, methanol, and other soluble hazardous substances. The barrier was constructed of a highly flexible, fiber-reinforced plastic with an air-inflated flotation collar, which supported the barrier, and a water-inflated seal, which sealed the barrier to the bottom of the waterway. The barrier was maintained in position by a mooring system.

As was described previously, a floating barrier with an open bottom was used in the model river to contain spilled materials; however, it failed to confine material long enough for effective biological treatment. Therefore, similar models were used.

The cylindrical barrier had a bottom area of 575 cm^2 and a water depth of 35 cm. The loss of contained material was thought to be due to bottom sediment sorption, seepage through the bottom sediment, leakage through the bottom seal, volatilization through the air-water interface, and so forth. These losses due to other than biological decomposition may be categorized into a first order reaction with respect to the substance concentration. When the water body inside a barrier is to be treated in a batch manner, the total mass removal rate can be expressed as:

$$\frac{dS}{dt} = -\frac{kXS}{K_s + S} - k_r S \qquad (124)$$

where k_r is the pollutant removal rate coefficient ($time^{-1}$) contributed by other than biological decomposition.

Use of a 575-cm^2 area of Lake Austin sediment, which had a geometric mean diameter of 0.173 mm with a geometric standard deviation of 1.70 mm, resulted in a k_r value of 0.00346 hr^{-1} for phenol. The value for methanol was observed to be 0.00873 hr^{-1} without aeration. The difference between the two coefficient values was believed to be due to methanol volatilization. When methanol was aerated through glass tubes with an air flow rate of 1 liter air/min/liter water, k_r increased to 0.00952 hr^{-1}.

When the mixing intensity within the barrier is not enough for complete suspension of the acclimated sludge as required for in situ batch treatment, sludge-containing cloth bags may be used. Then, Ek instead of k should be used in Equation 124, or:

$$\frac{dS}{dt} = -\frac{EkXS}{K_s + S} - k_r S . \qquad (125)$$

Then, S and X change with time and are obtained from Equations 81 and 82 with substitutions of Ek for k and k_r for k_e.

The confining barrier application tests were carried out using sludge-containing cloth bags as the acclimated bacterial source with phenol and methanol as a substrate, with groundwater flowing in the model river, and without any chemical aids at 28 °C. Oxygen was supplied at the cloth bag sites by means of aeration. The equivalent pH for phenol removal in the groundwater was 7.0 (see Table 10). Therefore, k at 28 °C is estimated to be 0.0724 hr^{-1} for phenol (see Figure 12). The average pH for methanol removal in the groundwater was 7.8 with an efficiency of 0.473 (see Table 16). Therefore, k for methanol at 28 °C is estimated to be 0.1143 hr^{-1} (=0.2656 x 0.91 x 0.473) (see Figure 25). Detailed experimental conditions and information necessary for the prediciton of S and X with aeration time are tabulated in Table 41.

At the end of two days of aeration, about 2% of the applied phenol sludge and about 5% of the applied methanol sludge initially leaked from the cloth bags. Theoretically computed substrate removal patterns of phenol and methanol, on the assumption that all the sludge stayed within the cloth bags, are compared to the observed substrate concentrations in Figures 68 and 69. The aeration times required to obtain a given substrate removal were observed to be approximately 5% less than those computed, probably due to organisms that escaped from the cloth bags.

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TABLE 41. EXPERIMENTAL CONDITIONS AND KINETIC INFORMATION FOR THE PHENOL AND METHANOL REMOVAL BATCH TESTS USING FIXED CONFINING BARRIERS AND SLUDGE-CONTAINING CLOTH BAGS

Parameters	Test substance Phenol	 Methanol
Temperature (°C)	28	28
Dilution water	Groundwater	Groundwater
Initial pH	8.6	8.6
Equivalent pH (or average pH)	7.0 (Table 10)	7.8 (Table 16)
Efficiency of dilution water	1.0 (Table 10)	0.473 (Table 16)
f-Factor	1.0 (Figure 12)	0.91 (Figure 25)
k (hr^{-1})	0.0724	0.1143
K_s (mg/l)	236	2,330
k_d (hr^{-1})	0.00672 (Eq. 41)	0.00291 (Eq. 58)
a	1.21	1.25
C (cm/hr)	0.684 (Table 18)	1.35 (Table 18)
Initial X_c (mg/l as VSS)	31,320	5,754
Initial X (mg/l as VSS)	1,350	635
Cloth bag shape factor, V_c/A_c (cm)	0.675	0.685

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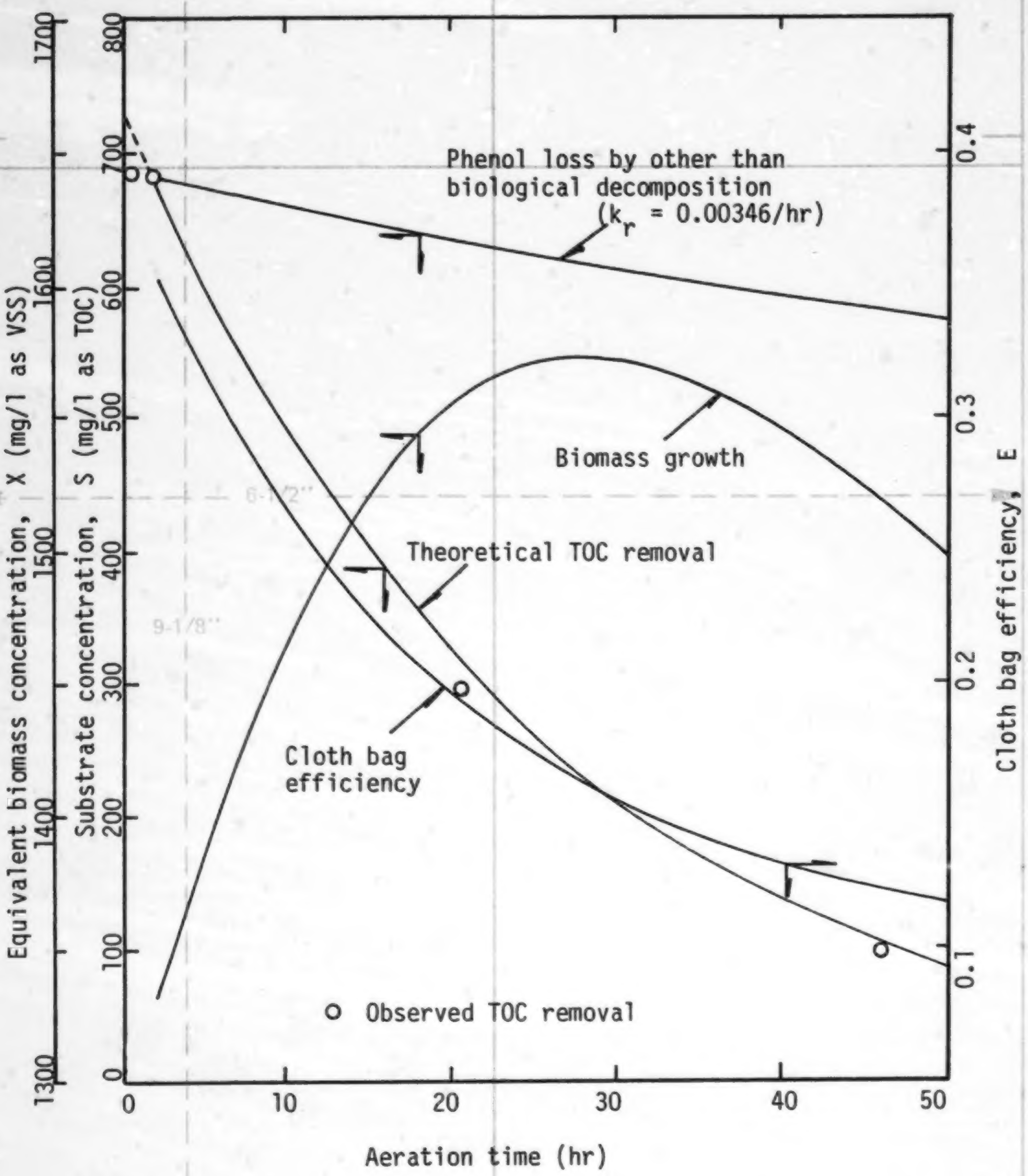

Figure 68. Phenol removal using a fixed barrier with sludge-containing cloth bags.

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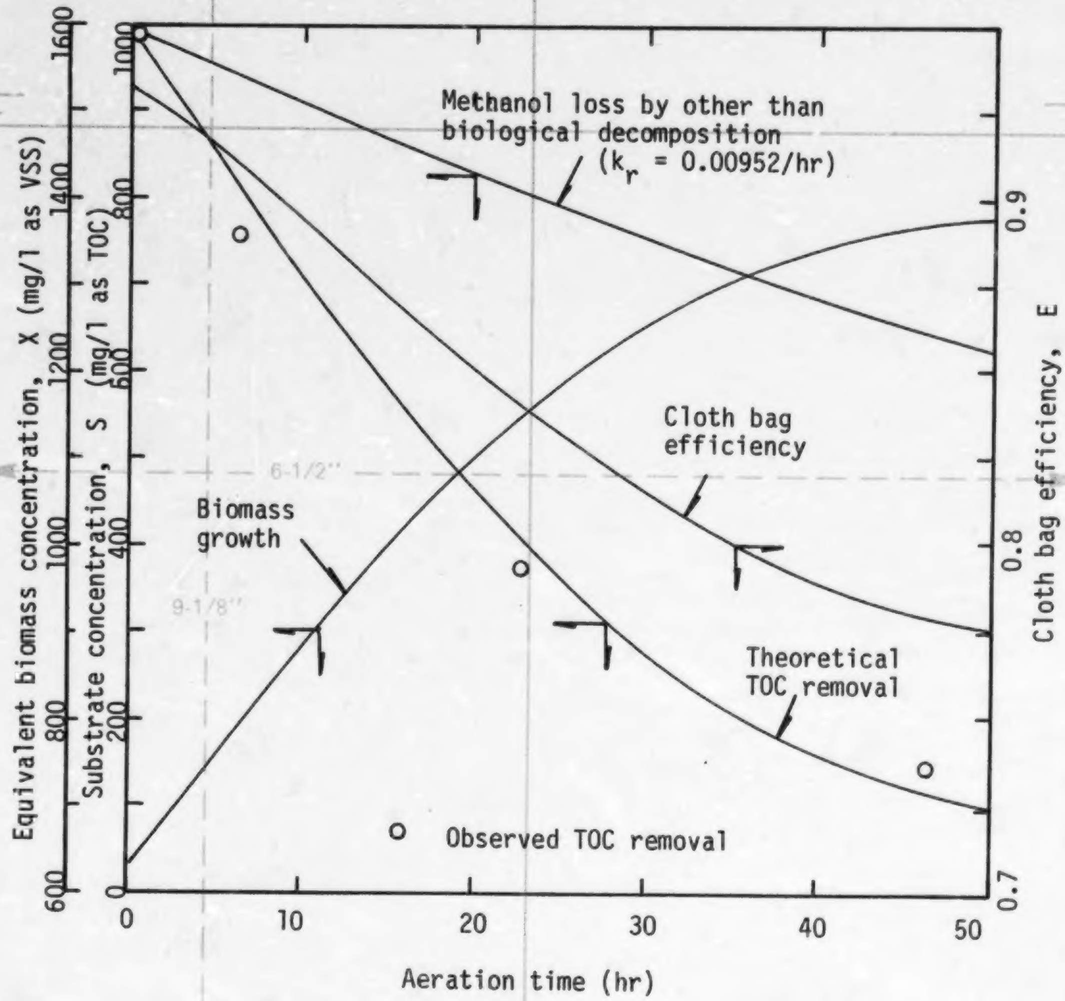

Figure 69. Methanol removal using a fixed barrier with sludge-containing cloth bags.

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REFERENCES

APHA, AWWA, and WPCF. 1970. *Standard Methods for the Examination of Water and Wastewater*, 13th. Ed. Amer. Pub. Health Assoc., Washington, D.C.

Anderson, B. G. 1944. "The Toxicity Thresholds of Various Substances Found in Industrial Wastes as Determined by the Use of *Daphnia magna*." Sewage Works J., 16: 1156-1165.

Anderson, B. G. 1960. "The Toxicity of Organic Insecticides to Daphnia." In: C. M. Tarzwell, *Biological Problems in Water Pollution*. Robert A. Taft San. Eng. Center Tech. Report W-60-3, pp. 94-95.

Anthony, C. and Zatman, L. J. 1965. "The Microbial Oxidation of Methanol. The Alcohol Dehydrogenase of *Pseudomonas sp. M27*." Biochem. 96: 808.

Anthony, C. and L. J. Zatman. 1967a. "The Microbial Oxidation of Methanol. The Purification and Properties of the Alcohol Dehydrogenase of *Pseudomonas sp. M27*." Biochem J. 104: 953.

Anthony, C. and Zatman, L. J. 1967b. "The Microbial Oxidation of Methanol. The Prosthetic Group of the Alcohol Dehydrogenase of *Pseudomonas sp. M27*." Biochem. J. 104: 960.

Applegate, V. C., J. H. Howell, and A. E. Hall, Jr. 1957. "Toxicity of 4346 Chemicals to Larval Lampreys and Fishes." U. S. Fish & Wildlife Service Special Scientific Report 207.

Bandt, J. H. 1953. "Acrylonitrile as an Effluent Poisoning to Fish." Zeitschrift fur Fischerei 2: 457-416.

Barnes, V. and V. Cerna. 1959. "Acute Toxicity and Mechanism of Action." J. Hyg., Epidemiol., and Microbiol. Immuno. 3: 106-116.

Batte, E. G., L. E. Swanson, and J. B. Murphy. 1951. "New Molluscicides for the Control of Freshwater Snails." Amer. Veterinary Res. 12: 158-160.

Bedford, J. W., E. W. Roelofs, and M. J. Zabik. 1968. "The Freshwater Mussel as a Biological Monitor of Pesticide Concentrations in a Lotic Environment. Limnol. & Oceanog. 13: 118-128.

Boni, P. 1965. "Acute Toxicity and Elimination of Phenol Injected into Fish (*Carassius auratus* L.)." Experimental 21: 222-223.

Boyd, C. E. and D. E. Ferguson. 1964. "Susceptibility and Resistance of Mosquito Fish to Several Insecticides." J. Econ. Entomol. 57: 430-431.

Brieger, H., F. Rieders, and W. A. Hodes. 1952. "Acrylonitrile: Spectrophotometric Determination, Acute Toxicity, and Mechanism of Action." Arch. Ind. Hyg. Occu. Med. 6: 128-140.

Bringmann, G. and R. Kuhn. 1959. "The Toxic Effects of Waste Water on Aquatic Bacteria, Algae and Small Crustaceans." Gesundheits-Ing 80: 115-120.

Brocksen, R. T. and H. T. Bailey. 1973. "Respiratory Response of Juvenile Chinook Salmon and Striped Bass Exposed to Benzene, A Water-Soluble Component of Crude Oil." Proc. Joint Conf. on Prevention and Control of Oil Spills, March 13-15, 1973.

Brown, L. R., R. J. Strawinski, and C. S. McCleskey 1964. "The Isolation and Characterization of Methanomonas methanooxidans, Can. J. Microbiol. 10: 79.

Brown, L. S. 1972. "A Physical Barrier System for Control of Hazardous Material Spills in Waterways." Proc. of the 1972 National Conf. on Control of Hazardous Material Spills, March 1972.

Brown, V. M., D. H. M. Jordan, and B. A. Tiller. 1967. "The Effect of Temperature on the Acute Toxicity of Phenol to Rainbow Trout in Hard Water." Water Research 1: 587-594.

Brown, V. M., D. G. Shurben, and J. K. Fawell. 1967. "The Acute Toxicity of Phenol to Rainbow Trout in Saline Waters." Water Research 1: 683-685.

Browning, E. 1950. "Industrial Solvents: The Aromatic and Cyclic Hydrocarbons." Brit. Med. J. 1: 19-23.

Browning, E. 1959. "Toxic Solvents: A Review." British J. Indus. Med. 16 23-39.

Bryant, M. P., S. F. Tzeng, I. M. Robinson, and A. E. Joyner, Jr. 1971. "Nutrient Requirements of Methanogic Bacteria." In Anaerobic Biological Treatment Processes, Edited by F. G. Pholand. Adv. in Chem. Ser. 105: 23-40. Amer. Chem. Soc., Washington, D. C.

Burdick, G. E., H. J. Dean, and E. J. Harris. 1958. "Toxicity of Cyanide to Brown Trout and Smallmouth Bass." NY Fish Game J. 5: 133-163. Cited in Water Quality Criteria Data Book 3, 1971.

Burnett, N. E. 1975. "The Effect of Salinity Variations on the Activated Sludge Process." Poll. Abst. 6: 347.

Butler, P. A. 1967. "Pesticide Residues in Estuarine Mollusks." Proc. of the Nat. Symp. on Estuarine Pollution, Stanford Univ. pp 107-121. Cited in Water Quality Criteria Data Book 3, 1971.

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Buzzell, J. C. Jr., C. H. Thompson, and D. W. Ryckman. 1969. "Behavior of Organic Chemicals in the Aquatic Environment. Part III - Behavior in Aerobic Treatment Systems (Activated Sludge)." Manu. Chem. Assoc., Washington, D.C.

Buzzell, J. C., Jr., R. H. F. Young, and D. W. Ryckman. 1968. "Behavior of Organic Chemicals in the Aquatic Environment Part II - Behavior in Dilute Systems." Manu. Chem. Assoc. Washington, D. C.

Cairns, J. Jr. and A. Scheier. 1959. "The Relationship of Bluegill Sunfish Body Size to Tolerance for some Common Chemicals." Proc. 13th Ind. Waste Con., Purdue Univ. Eng. Ext. Ser. 96: 243-252.

Cairns, J. Jr. and A. Scheier. 1968. "A Comparison of the Toxicity of Some Common Industrial Waste Components Tested Individually and Combined." Progressive Fish Culture 30: 3-8.

Camp, T. R. and R. L. Meserve. 1974. Water and Its Impurities, 2nd. ed. Dowden, Hutchinson & Ross, Inc. PA p. 332.

Carpenter, W. L. et al. 1968. "Temperature Relationship in Aerobic Treatment and Disposal of Pulp and Paper Wastes." J. WPCF 40: 733.

Casper, V. L. 1967. "Galveston Bay Pesticide Study -Water and Oyster Samples Analyzed for Pesticide Residues Following Mosquito Control Program." Pesticide Monitoring J. 1 (3): 1315. Cited in Water Quality Criteria Data Book 3, 1971.

Chambers, C. W., H. H. Tabak, and P. W. Kabler. 1963. "Degradation of Aromatic Compounds by Phenol-Adapted Bacteria." J. WPCF 35: 1517-1528.

Chapman, R. J. 1972. "An Outline of Reaction Sequences Used for the Bacterial Degradation of Phenolic Compounds." In: Degradation of Synthetic Organic Molecules in the Biosphere. National Academy of Sciences, Washington, D. C. pp 17-55.

Chearn, D. G. and S. P. Myers (Eds.) 1973. "The Microbial Degradation of Oil Pollutants." Center for Wetland Resources, Louisiana State Univ. Baton Rouge, LA., Publ, No. LSU-SG-73-01.

Chen, C. W. and R. E. Selleck. 1968. "A Kinetic Model of Fish Toxicity Threshold." 41st WPCF Conf., Chicago. IL Cited in Water Quality Criteria Data Book 3, 1971.

Cherry, A. B., A. J. Gabaccia, and H. W. Senn. 1956. "The Assimilation Behavior of Certain Toxic Organic Compounds in Natural Water." Sewage and Ind. Wastes 28: 1137-1146.

Clemens, H. P. and K. E. Sneed. 1959. "Lethal Doses of Several Commercial Chemicals for Fingerling Channel Catfish." U. S. Fish and Wildlife Service Spec. Scien. Rep. 316. Cited in Water Quality Criteria Data Book 3, 1971.

PAGE NUMBER
EPA Form 2350-4 (4-80)
(PREVIOUSLY CIN. EPA FORM 287)
TYPING GUIDE SHEET

Clendenning, K. A. and W. J. North. 1960. "Effects of Wastes on the Giant Kelp, Macrocystis pyrifera." Proc. of 1st Internat. Con. on Waste Disposal in the Marine Environ. pp. 32-91. Cited in Water Quality Criteria Data Book 3, 1971.

Cohen, J. M., L. J. Kamphake, A. E. Lemke, C. Henderson, and R. L. Woodward. 1961. "Effect of Fish Poisons on Water Supplies. Part 1-Removal of Toxic Materials." J. Amer. Water Works Assoc. 52: 1551-1556. Cited in Water Quality Criteria Data Book 3, 1971.

Cole, W. H. and J. B. Allison. 1930. "Chemical Stimulation by Alcohols in the Barnacle, the Frog, and Planaria." J. General Physiol. 14: 17-86.

Cope, O. B. 1963, "Sport Fishery Investigations, 1961-62." U.S. Fish and Wildlife Service Circular 167: 26-42.

Cope, O. B. 1965. "Sport Fishery Investigations, 1964." U. S. Fish and Wildlife Service Circular 226: 51-63. Cited in Water Quality Criteria Data Book 3, 1971.

Cope, O. B. 1966. "Contamination of the Freshwater Ecosystem by Pesticides." J. Appl. Ecol. 3 (Suppl): 33-44.

Council on Environmental Quality. 1975. "National Oil and Hazardous Substances Pollution Contingency Plan." Federal Register, Vo. 40, No. 28, Part II, 6282, Washington, D. C.

Coulter, K. E., H. Kehde, and B. F. Hiscock. 1967. "Styrene." In: Kirk Othmer Encyclopedia of Chemical Technology. Interscience Publishers, NY 19: 55-85.

Courtenay, W. R., Jr. and M. H. Roberts, Jr. 1973. "Environmental Effects on Toxaphene Toxicity to Selected Fishes and Crustaceans." EPA-R3-73-035, Ecol. Res. Ser., Office of Research and Monitoring, U. S. Environmental Protection Agency, Washington, D. C.

Crosby, D. G. 1972. "Environmental Photooxidation of Pesticides." In: Degradation of Synthetic Organic Molecules in the Biosphere. National Academy of Sciences, Washington, D. C. pp 200-290.

Currier, H. B. 1951. "Herbicidal Properties of Benzene and Certain Methyl Derivatives." Hilgardia 20: 383-406.

Cushing, C. E. and J. R. Olive. 1957. "Effects of Toxaphene and Rotenone Upon the Macroscopic Bottom Fauna of Two Northern Colorado Reservoirs." Trans. Amer. Fishery Soc. 86: 294-301. Cited in Water Quality Criteria Data Book 3, 1971.

Czaikowska, T. 1971. "Excretion of Certain Metabolites Upon Single Administration of Acrylonitrile." Med. Practice 22: 381-385.

Dagley, S. 1972. "Microbial Degradation of Stable Chemical Structures: General Features of Metabolic Pathways." In: Degradation of Synthetic Organic Molecules in the Biosphere. National Academy of Sciences, Washington, D. C. pp 1-16.

Davis, C. V. 1952. Handbook of Applied Hydraulics, 2nd. ed. McGraw-Hill Book Co., Inc., NY p. 1204.

Dawson, G. W., A. J. Shuckrow, and B. W. Mercer. 1972. "Strategy for Treatment of Waters Contaminated by Hazardous Materials." Proc. of the 1972 Nat. Conf. on Control of Hazardous Material Spills. March 1972, Houston, Texas.

Dawson, G. M., A. J. Shuckrow, and W. H. Swift. 1970. "Control of Spillage of Hazardous Polluting Substances." Report to the Federal Water Quality Administration by the Battelle Memorial Institute, Richland, Washington. Water Pollution Control Res. Ser. Report No. 15090 FOZ 10/70.

Dean, J. A. 1973. Lang's Handbook of Chemistry, 11th. ed. McGraw-Hill Book Co., Inc., NY.

Dethier, V. G. and L. E. Chadwick. 1947. "Rejection Thresholds of the Blowfly for a Series of Aliphatic Alcohols." J. General Physiol. 30: 247-253.

Dickerson, B. W., C. J. Campbell, and M. Stankard. 1955. "Further Operating Experiences on Biological Purification of Formaldehyde Wastes." Proc. 9th Ind. Waste Conf., Purdue University. Eng. Ext. Ser. 87: 331-351.

Dowden, B. F. and H. J. Bennett. 1965. "Toxicity of Selected Chemicals to Certain Animals." J. WPCF 37: 1308-1316.

Dworkin, M. and J. W. Forster. 1956. "Studies on Pseudomonas methanica." J. Bacteriol. 72: 646.

Eckenfelder, W. W. Jr. 1966. Industrial Water Pollution Control. McGraw-Hill, NY.

Eckenfelder, W. W., Jr. 1967. "Theory of Biological Treatment of Trade Wastes." J. WPCF 39: 240.

Eckenfelder, W. W., Jr. 1970. Water Quality Engineering for Practicing Engineers. Barnes and Noble, Inc., NY.

Eckenfelder, W. W., Jr. and D. L. Ford. 1970. Water Pollution Control: Experimental Procedures for Process Design. The Pemberton Press, Jenkins Publishing Co., Austin, Texas.

Eckenfelder, W. W., Jr. and D. J. O'Connor. 1961. Biological Waste Treatment. Pergamon Press, NY.

Eckenfelder, W. W., Jr., R. Kleffman, and J. Walker. 1957. "Some Theoretical Aspects of Solvent Stripping and Aeration of Industrial Wastes." Proc. 11th. Ind. Waste Conf., Purdue Univ. Ext. Ser. 91: 14.

Engelbrecht, R. S., A. F. Gaudy, J. M. Cederstand, 1961. "Diffused Air Stripping of Volatile Components of Petrochemical Wastes." J. WPCF 33: 127.

Evans, W. C. 1947. "Oxidation of Phenol and Benzoic Acid by Some Soil Bacteria." Biochem. J. 41: 373-382.

Fair, G. M., J. C. Geyer, and D. A. Okun. 1968. Water and Wastewater Engineering, Vol. 2. John Wiley & Sons, Inc., NY pp 22-23.

Ferguson, D. E., W. D. Cotton, D. T. Gardner, and D. D. Culley. 1965. "Tolerances to Five Chlorinated Hydrocarbon Insecticides in Two Species of Fish From a Transect of the Lower Mississippi River." J. Mississippi Acad. Sci. 11: 235-237. Cited in Water Quality Criteria Data Book 3, 1971.

Ferguson, D. E., D. D. Culley, W. D. Cotton, and R. P. Dodds. 1964. "Resistance to Chlorinated Hydrocarbon Insecticides in Three Species of Freshwater Fish." Bio Science 14: 43-44.

Ferguson, D. E., D. D. Culley, W. D. Cotton, and R. P. Dodds. 1965. "Tolerances of Two Populations of Fresh Water Shrimp to Five Chlorinated Hydrocarbon Insecticides." J. Mississippi Acad. Sci. 11: 235-237. Cited in Water Quality Criteria Data Book 3, 1971.

Fischer, H. B. 1966. "A Note on the One-Dimensional Dispersion Model." Air & Water Pollut. Int. J. 10: 443.

Fukano, K. G. and F. F. Hooper. 1958. "Toxaphene as a Selective Fish Poison." Progressive Fish Culture 20: 189-190. Cited in Water Quality Criteria Data Book 3, 1971.

Gakstatter, J. H. 1968. "Rates of Accumulation of ^{14}C-Dieldrin Residues in Tissues of Goldfish Exposed to a Single Sublethal Dose of ^{14}C-Aldrin." J. Fish. Res. Bd. Can. 25: 1797-1801.

Garrett, J. T. 1957. "Toxicity Considerations in Pollution Control." Industrial Wastes 2: 17-19.

Gaudy, A. F., Jr. and R. S. Engelbrecht. 1960. "Basic Biochemical Considerations During Metabolism in Growing vs. Respiring System." 3rd. Biol. Waste Treat. Conf., Manhattan College, NY.

Gaudy, A. F., Jr. and R. S. Englebrecht. 1961. "Quantiative and Qualitative Shock Loading of Activated Sludge Systems." J. WPCF 33: 805.

Gaudy, A. F., R. S. Engelbrecht, and B. G. Turner. 1961. "Stripping Kinetics of Volatile Components of Petrochemical Wastes." J. WPCF 33: 382.

Gaufin, A. R., L. D. Jensen, A. V. Nebeker, T. Nelson, and R. W. Teel. 1965. "The Toxicity of Ten Organic Insecticides to Various Aquatic Invertebrates." Water and Sewage Works 112: 276-279. Cited in Water Quality Criteria Data Book 3, 1971.

Georgacakis, E. and M. A. Q. Khan. 1971. "Toxicity of the Photoisomers of Cyclodiene Insecticides to Freshwater Animals." Nature 233: 120-121.

Gersdorff, W. A. 1943. "Effect of Introducing the Carboxyl Group into the Phenol Molecule on Toxicity to Goldfish." Amer. J. Pharm. 115: 159-167.

Gersdorff, W. A. and L. E. Smith. 1940. "Effect of Introduction of the Halogens into the Phenol Molecule on Toxicity to Goldfish, I. Monochlorophenols." Amer. J. Pharmacy 112: 197-204.

Gibson, D. T. 1968. "Microbial Degradation of Aromatic Compounds." Science 161: 1093-1097.

Gibson, D. T. 1972. "Initial Reactions in the Degradation of Aromatic Hydrocarbons." In: Degradation of Synthetic Organic Molecules in the Biosphere. National Academy of Sciences, Washington, D. C. 116-136.

Gillette, L. A., D. L. Miller, and H. E. Redman. 1952. "Appraisal of a Chemical Waste Problem by Fish Toxicity Tests." Sewage and Ind. Wastes 24: 1397-1401.

Harris, E. K. 1963. "A New Statistical Approach to the One-Dimensional Diffusion Model." Air & Water Pollut. Int. J. 7: 799.

Hashimoto, K. and R. Kanai. 1965. "Toxicology of Acrylonitrile: Metabolism, Mode of Action, and Therapy." Indus. Health (Japan) 3: 30-46.

Hashimoto, K. and R. Kanai. 1972. "Effect of Acrylonitrile on Sulphydryls and Pyruvate Metabolism in Tissues." Biochem. Pharm. 21: 635-640.

Hatfield, R. 1957. "Biological Oxidation of Some Organic Compounds." Ind. & Eng. Chem. 49: 192-196.

Helmers, E. N., J. D. Frame, A. E. Greenberg, and C. N. Sawyer, 1951. "Nutritional Requirements in the Biological Stabilization of Industrial Wastes. II. Treatment with Domestic Sewage." Sewage and Ind. Wastes 23: 884.

Helmers, E. N., J. D. Frame, A. E. Greenberg, and C. N. Sawyer. 1952. "Nutritional Requirements in the Biological Stabilization of Industrial Wastes. III. Treatment with Supplementary Nutrients." Sewage and Ind. Wastes 24: 496.

Hemphill, J. E. 1954. "Toxaphene as a Fish Toxin." Progressive Fish Culture 16: 41-42.

Henderson, C., Q. H. Pickering, and A. E. Lemke. 1961. "The Effect of Some Organic Cyanides (Nitriles) on Fish." Proc. 15th Indus. Waste Conf. Purdue University, Eng. Bull. 45: 120-130.

Henderson, C., Q. H. Pickering, and C. M. Tarzwell. 1959. "Relative Toxicity of Ten Chlorinated Hydrocarbon Insecticides to Four Species of Fish." Trans. Amer. Fish. Soc. 88: 23-32.

Hendrick, R. D. and T. R. Everett. 1965. "Toxicity to the Louisiana Red Crawfish of Some Pesticides Used in Rice Culture." J. Econ. Entomol. 58: 958-961.

Henegar, D. L. 1966. "Minimum Lethal Levels of Toxaphene as a Piscicide in North Dakota Lakes." U. S. Fish and Wildlife Service, Bureau of Sport Fisheries and Wildlife Resource Publ. 7. Cited in Water Quality Criteria Date Book 3, 1971.

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TYPING GUIDE SHEET

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TOP OF IMAGE AREA
DROPPED HEAD: BEGIN SECTIONS HERE

Heptinstall, J. and J. R. Quayle. 1970. "Pathways Leading to and from Serine during Growth of Pseudomonas AM 1 on C_1 Compounds or Succinate." Biochem. J. 117: 563.

Hermann, E. R. 1959. "A Toxicity Index for Industrial Wastes." Indus. and Eng. Chem. 51: 84A-87A.

Heukelekian, H., H. E. Orford, and R. Manganelli. 1951. "Factors Affecting the Quantity of Sludge Production in the Activated Sludge Process." Sewage and Ind. Wastes 23: 8.

Hill, D. W. and P. L. McCarty. 1967. "Anaerobic Degradation of Selected Chlorinated Hydrocarbon Pesticides." J. WPCF 39: 1259-1277.

Hilsenhoff, W. L. 1965. "The Effect of Toxaphene on the Benthos in a Thermally-Stratified Lake." Trans. Amer. Fish. Soc. 94: 210-213.

Hodgson, E. S. 1951. "Reaction Thresholds of an Aquatic Beetle, Laccophilus maculosus, to Salts and Alcohols." Physiol. Zool. 24: 131-140.

Hoffman, D. A. and J. R. Olive. 1961. "The Effects of Rotenone and Toxaphene Upon Plankton of Two Colorado Reservoirs." Limnol. & Oceanography 6: 219-222

Hooper. F. F. and A. R. Grzenda. 1955. "The Use of Toxaphene as a Fish Poison." Trans. Amer. Fish. Soc. 85: 180-190. Cited in Water Quality Criteria Data Book 3, 1971.

Howland, W. 1958. "Flow over Porous Media as in a Trickling Filter." Proc. 12th. Ind. Waste Conf., Purdue Univ. 94: 435.

Hubault, E. 1936. "The Toxicity of Hydrocarbons Towards Freshwater Fish." C. R. Acad. Agric. (France) 22: 130.

Hughes, R. A. and G. F. Lee. 1973. "Toxaphene Accumulation in Fish in Lakes Treated for Rough Fish Control." Environ. Sci. and Technol. 7: 934-939.

Huibregtse, K. R., R. C. Scholz, R. E. Wullschleger, C.A. Hanson, and I. Wilder. 1976. "Users Manual for Treating Hazardous Spills." Proc. of the 1976 National Conf. on Control of Hazardouus Material Spills, New Orleans, LA.

Huish, M. T. 1961. "Toxaphene as a Fish Eradicant in Florida." 15th Ann. Conf., Southeastern Assoc. Game & Fish Comm. pp 200-205. Cited in Water Quality Criteria Data Book 3, 1971.

Jensen, L. D. and A. R. Gaufin. 1964. "Effects of Ten Organic Insecticides on Two Species of Stonefly Naiads." Trans. Amer. Fish. Soc. 93: 27-34.

Jensen, L. D. and A. R. Gaufin. 1966. "Acute and Longterm Effects of Organic Insecticides on Two Species of Stonefly Naiads." J. WPCF 38: 1273-1286.

Johnson, W. C. 1966. "Toxaphene Treatment of Big Bear Lake, California." Calif. Fish and Game 52: 173-179.

BEGIN LAST LINE OF TEXT
BOTTOM OF IMAGE AREA; OUTSIDE DIMENSION FOR TABLES AND ILLUSTRATIONS

PAGE NUMBER
EPA Form 2350-4 (4-80)
(PREVIOUSLY CIN. EPA FORM 287)
TYPING GUIDE SHEET

BEGIN FIRST LINE OF TEXT HERE
CENTER OF PAGE
TOP OF IMAGE AREA
DROPPED HEAD: BEGIN SECTIONS HERE

Jones, J. R. E. 1951. "The Reactions of the Minnow, *Phoxinus phoxinus*, to Solutions of Phenol, Ortho-Cresol and Para-Cresol." J. Exp. Biol. *28*: 261-270.

Kallman, B. J., O. B. Cope, and R. J. Navarre. 1962. "Distribution and Detoxification of Toxaphene in Clayton Lake, New Mexico." Trans. Amer. Fish. Soc. *91*: 14-22.

Katz, M. 1961. "Acute Toxicity of Some Organic Insecticides to Three Species of Salmonids and to the Threespine Stickleback." Trans. Amer. Fish. Soc. *90*: 264-268.

Kauss, P., T. C. Hutchinson, C. Soto, J. Hellebust, and M. Griffiths. 1973. "The Toxicity of Crude Oil and Its Components to Freshwater Algae." Proc. of Joint Conference on Prevention and Control of Oil Spills, Washington, D. C.

Kearney, P. C. and D. D. Kaufman. 1972. "Microbial Degradation of Some Chlorinated Pesticides." In: *Degradation of Synthetic Organic Molecules in The Biosphere*. National Academy of Sciences, Washington, D. C. pp. 166-189.

Keyna, D. 1940. "The Toxic Action of Various Substances on Algae, In Particular, *Ankistrodesmus* and *Scenedesmus*." Kl, Mit. Ver. Wasser-Bodenand Lufthyg *16*: 67.

Kittredge, J. S. 1971. "Effects of the Water-Soluble Components of Oil Pollution on Chemoreception by Crabs." NTIS Publication AD-738, 505.

Knowles, F. L., W. V. Parker, and H. A. Johnson. 1941. "Observations on the Use of 'Phenol' Larvacides for Mosquito Control." Public Health Rep. *56*: 1627-1641.

Krieger, R. I. and P. W. Lee. 1973. "Inhibition of In Vivo and In Vitro Epoxidation of Aldrin and Potentiation of Toxicity of Various Insecticide Chemicals by Diquat in Two Species of Fish." Arch. Environ. Contam. and Toxicol. *1*: 112-121.

Krombach, H. and J. Barthel. 1963. "Investigation of a Small Watercourse Accidentally Polluted by Phenol Compounds." Int. J. Air and Water Poll. *7*: 39-46.

Kudo, A. and E. F. Gloyna. 1969. "Radioactivity Transport in Water--Interaction between Flowing Water and Bed Sediments." Tech. Report to the Atomic Energy Comm. Contract AT-(11-1)-490.

Laham, S. 1970. "Metabolism of Industrial Solvents. I. The Biotransformation of Benzene and Benzene Substitutes." Occu. Health Rev. *21*: 24-28.

Lammering, M. W. and N. C. Burbank, Jr. 1961. "The Toxicity of Phenol, σ-Chlorophenol and -Nitrophenol to Bluegill Sunfish." Proc. 15th Ind. Waste Con., Purdue University, Eng. Bull *106*: 541-555.

Lank, J. C., Jr. and A. T. Wallace. 1970. "Effect of Acrylonitrile on Anaerobic Digestion of Domestic Sludge." 25th Ind. Waste Conf. Pt. Two, Purdue University, Eng. Ext. Ser. *137*: 518-527.

BEGIN LAST LINE OF TEXT
BOTTOM OF IMAGE AREA; OUTSIDE DIMENSION FOR TABLES AND ILLUSTRATIONS
3/8"

PAGE NUMBER
EPA Form 2350-4 (4-80)
(PREVIOUSLY CIN. EPA FORM 287)
TYPING GUIDE SHEET

Lawrence, H. M. 1950. "Toxicity of Some New Insecticides to Several Species of Pondfish." Progressive Fish Culture 12: 141-146.

Lawton, A. H., T. R. Sweeney, and H. C. Dudley. 1943. "Toxicity of Acrylonitrile (Vinyl Cyanide). III. Determination of Thiocyanates in Blood and Urine." J. of Ind. Hyg. & Toxicol. 25: 13-19.

Leshniowsky, W. O., P. R. Dugan, R. M. Pfister, J. I. Frea, and C. I. Randles. 1970. "Aldrin: Removal from Lake Water by Flocculent Bacteria." Science 169: 993-995.

Lichtenstein, E. P. 1972. "Environmental Factors Affecting Fate of Pesticides." In: Degradation of Synthetic Organic Molecules in the Biosphere, National Academy of Sciences, Washington, D. C., pp. 190-205.

Liepolt, R. 1953. "Fish Poisoning by Waste Waters in the Murz." Ost Wasser W. 5: 56.

Lloyd, R. 1961. "Effect of Dissolved Oxygen Concentrations on the Toxicity of Several Poisons to Rainbow Trout (Salmo gairdnerii)." J. Exp. Biol. 38: 447-455.

Ludzak, F. J. and M. B. Ettinger. 1960. "Chemical Structures Resistant to Aerobic Biochemical Stabilization," J. WPCF 32: 1173-1200.

Ludzak, F. J., R. B. Schaffer, and M. B. Ettinger. 1959. "Biochemical Oxidation of Some Commercially Important Organic Cyanides." Sewage and Ind. Wastes 31: 3344.

Lutin, P. A. 1970. "Removal of Organic Nitriles from Wastewater Systems." J. WPCF 42: 1632-1642.

Mahdi, M. A. 1966. "Mortality of Some Species of Fish to Toxaphene at Three Temperatures." U. S. Fish and Wildlife Service, Bureau Sport Fisheries and Wildlife Resource Publ. 10.

Malaney, G. W. 1960. "Oxidative Abilities of Aniline - Acclimated Activated Sludge." J. WPCF 32: 1300-1311.

Malaney, G. W. and R. E. McKinney. 1966. "Oxidative Abilities of Benzene Acclimated Activated Sludge." Water & Sewage Works 113: 302-309.

Marchetti, R. 1965. "The Toxicity of Nonyl Phenol Ethoxylate to the Developmental Stages of the Rainbow Trout, Salmo gairdnerii." Ann. Appl. Biol. 55: 425-430.

Marion, C. V. and G. W. Malaney. 1963. "The Oxidation of Aliphatic Compounds by Alcaligenes faecalis." J. WPCF 35: 1269-1284.

Marr, E. K. and R. W. Stone. 1961. "Bacterial Oxidation of Benzene." J. Bacteriol. 81: 425-430.

McAuliffe, C. 1963. "Solubility in Water of C_1 -C_9 Hydrocarbons." Nature 200: 1092-1093.

BEGIN FIRST LINE OF TEXT HERE
CENTER OF PAGE
TOP OF IMAGE AREA
DROPPED HEAD; BEGIN SECTIONS HERE

McCarty, P. L. 1964. "Thermodynamics of Biological Synthesis and Growth." 2nd. Int. Conf. on Water Pollution Res., Tokyo, Japan.

McDonald, S. 1962. "Rapid Detection of Chlorinated Hydrocarbon Insecticides in Aqueous Suspension with Gammarus lacustris." Can. J. Zool. 40: 719723.

McKee, J. E. and H. W. Wolfe 1963. Water Quality Criteria. Calif. Water Qual. Bd. Publ. 3-A, Second Edition.

McKenna, E. J. 1972. "Microbial Metabolism of Normal and Branched Chain Alkanes." In: Degradation of Synthetic Organic Molecules in the Biosphere, National Academy of Science, Washington, D. C. pp. 73-97.

McKinney, R. E. 1962. Microbiology for Sanitary Engineers. McGraw-Hill Book Co., Inc., NY.

McKinney, R. E. and J. S. Jeris. 1955. "Metabolism of Low Molecular Weight Alcohols by Activated Sludge." Sewage and Ind. Wastes 27: 728-735.

McKinney, R. E., H. D. Tomlinson, and R. L. Wilcox. 1956. "Metabolism of Aromatic Compounds by Activated Sludge." Sewage and Ind. Wastes 28: 547-557.

Meehan, W. R. and W. L. Sheridan. 1966. "Effects of Toxaphene on Fishes and Bottom Fauna of Big Kitoi Creek, Afognak Island, Alaska." U. S. Fish and Wildlife Service, Bureau Sport Fisheries and Wildlife Resource Publ. 12.

Metcalf & Eddy, Inc. 1972. Wastewater Engineering, McGraw-Hill Book Co., Inc. NY.

Mills, E. J., Jr. and V. T. Stack, Jr. 1955. "Acclimation of Microorganisms for the Oxidation of Pure Organic Chemicals." Proc. 9th Ind. Waste Conf., Purdue Univ. Eng. Ext. Ser. 87: 449-464.

Mitrovic, V. V., V. M. Brown, D. G. Shurben, and M. H. Berryman. 1968. "Some Pathological Effects of Sub-Acute and Acute Poisoning of Rainbow Trout by Phenol." Water Research 2: 249-254.

Monod, J. 1949. "The Growth of Bacterial Cultures." Ann. Rev. of Microbiol. Vol. III.

Moore, S. L. and S. R. Kin. 1969. "Train Wreck Causes Cyanide Pollution." Water and Sewage Works 116: 35-40.

Movsumzade, K. M. 1970. "State of Free Amino Acids in the Brain and Liver of a Rabbit Under the Effects of Acrylonitrile." Izv Akad Nauk Azerb USSR, Ser Biol Nauk 4: 128-132.

Moye, W. C. and W. H. Luckmann. 1964. "Fluctuations in Populations of Certain Aquatic Insects Following Application of Aldrin Granules to Sugar Creek, Iroquois County, Illinois." J. Econ. Entomol. 57: 318-322.

Mulla, M. S., L. W. Isaak, and H. Axelrod. 1963. "Field Studies on the Effects of Insecticides on Some Aquatic Wildlife Species." J. Econ. Entomol. 56: 184-188.

BEGIN LAST LINE OF TEXT
BOTTOM OF IMAGE AREA; OUTSIDE DIMENSION FOR TABLES AND ILLUSTRATIONS
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PAGE NUMBER
EPA Form 2350-4 (4-80)
(PREVIOUSLY CIN. EPA FORM 257)
TYPING GUIDE SHEET

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TOP OF IMAGE AREA

Nebeker, A. V. and A. R. Gaufin. 1964. "Bioassays to Determine Pesticide Toxicity to the Amphipod Crustacean, Gammarus lacustris." Proc. Utah Acad. Sci. 41: 64-67.

Needham, R. G. 1966. "Effects of Toxaphene on Plankton and Aquatic Invertebrates in North Dakota Lakes." U. S. Fish and Wildlife Service, Bureau of Sport Fisheries and Wildlife Resource Publ. 8.

North, W. N., K. A. Clendenning, and H. L. Scotten. 1959. "The Effects of Waste Discharges Upon Kelp." Quarterly Progress Report (July-Sept. 1959), Calif. Institute of Marine Resources, Reference 60-4.

Novak, J. T. 1974. "Temperature - Substrate Interactions in Biological Treatment." J. WPCF 46: 1984.

Nozaka, J. and M. Kusunose. 1968. "Metabolism of Hydrocarbons in Micro-organisms, Part I. Oxidation of p-Xylene and Toluene by Cell-Free Enzyme Preparations of Pseudomonas aeruginosa." Agric. Biol. Chem. 32: 1033-1039.

Oginsky, E. L. and W. W. Umbreit. 1954. An Introduction to Bacterial Physiology W. H. Freeman Co., San Francisco, CA.

Patel, R. N. and D. S. Hoare. 1971. "Physiological Studies of Methane and Methanol - Oxidizing Bacteria: Oxidation of C-1 Compounds by Methylococcus capulatus." J. Bacteriol. 107: 187.

Patrick, R., J. Cairns, Jr., and A. Scheier. 1968. "The Relative Sensitivity of Diatoms, Snails and Fish to Twenty Common Constituents of Industrial Wastes." Progressive Fish Culture 30: 137-140.

Paulet, G. and J. Desnos. 1961. "Acrylonitrile: Toxicity, Mechanism of Action, and Therapeutic Uses." Arch. Intern. Pharmacodyn. 131: 54-83.

Paulet, G., J. Desnos, and J. Battig. 1966. "Toxicity of Acrylonitrile." Arch. Mal. Prof. Med. Trav., Secur. Sec. 27 (12): 849-856.

Pearson, E. A. 1968. "Kinetics of Biological Treatment." In: Advances in Water Quality Improvement, Edited by E. F. Gloyna and W. W. Eckenfelder, Jr. University of Texas Press, Austin, TX.

Phelps, E. E. 1944. Stream Sanitation, Wiley & Sons, Inc. NY.

Pickering, Q. H. and C. Henderson. 1966. "Acute Toxicity of Some Important Petrochemicals to Fish." J. WPCF 38: 1419-1429.

Placak, O. R. and C. C. Ruchhoft. 1947. "Studies of Sewage Purification. XVII. The Utilization of Organic Substrates by Activated Sludge." Sewage Works J. 19: 423- 440.

Poorman, A. E. 1973. "Effects of Pesticides on Euglena gracilis. I. Growth Studies." Bull. Environ. Contam. and Toxicol. 10: 25-28.

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EPA Form 2350-4 (4-80)
(PREVIOUSLY CIN. EPA FORM 287)
TYPING GUIDE SHEET

Powers, E. B. 1918. "The Goldfish as a Test Animal in the Study of Toxicity." Ill. Biol. Monogr. 4: 123-193.

Profitt, M. A. 1966. "Some Factors Affecting the Toxicity of Aldrin to Fishes." Proc. Indiana Acad. Sci. 75: 325-329.

Randall, C. W., H. R. Edwards, and P. H. King, 1972. "Microbial Process for Acidic Low-Nitrogen Wastes." J. WPCF 44: 401.

Reich, K. 1955. "The Effect of Cyanide and Azide on the Respiration of the Amoeba, *Mayorella palestinensis*." Physiol. Zool. 28: 145-151.

Renn, C. E. 1955. "Biological Properties and Behaviors of Cyanogenic Wastes." Sewage and Ind. Wastes 27: 297-310.

Rose, E. T. 1958. "Further Notes on Toxaphene in Fish Population Control." Iowa State Conservation Commission, Fish & Game Division, Quarterly Biol. Report 10: 5-7.

Rose, J. D. and D. J. Sutherland. 1967. "The Nature and Toxicity of the Photoconversion Products of Aldrin." Bull. Environ. Contam. and Toxicol. 2: 109.

Royer, L. M. 1966. "Bioassay Method for the Determination of Toxaphene in Lake Water." J. Fish. Res. Bd. Can. 23: 723-727.

Sanders, H. O. and O. B. Cope. 1966. "Toxicities of Several Pesticides to Two Species of Cladocerans." Trans. Amer. Fish. Soc. 95: 165-169.

Sanders, H. O. and O. B. Cope. 1968. "The Relative Toxicities of Several Pesticides to Naiads of Three Species of Stoneflies." Limnol. & Oceanog. 13: 112-117.

Sawyer, C. N. 1955. "Bacterial Nutrition and Synthesis." Proc. Conf. on Biol. Waste Treatment, Manhattan College, NY.

Sawyer, C. N. and P. L. McCarty. 1967. *Chemistry for Sanitary Engineers*. 2nd. Ed. McGraw-Hill Book Co. Inc., NY.

Schaumberg, F. D., T. E. Howard, and C. C. Walden. 1967. "A Method to Evaluate the Effects of Water Pollutants on Fish Respiration." Water Research 1 (10): 731-737.

Schoettger, R. A. and J. R. Olive. 1961. "Accumulation of Toxaphene by Fish-Food Organisms." Limnol. & Oceanog. 6: 216-219.

Schroepfer, G. J., M. L. Robins, and R. H. Susag, 1960. "A Reappraisal of Deoxygenation Rates of Raw Sewage, Effluents, and Receiving Waters." J. WPCF 32: 1212.

Servizi, J. A. and R. H. Bogan. 1963. "Free Energy as a Parameter in Biological Treatment." Proc. ASCE 89 (SA3): 17.

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Shelford, V. E. 1917. "An Experimental Study of the Effects of Gas Waste Upon Fishes, with Special Reference to Stream Pollution." Bull. IU. Lab. Nat. Hist. XI: 381-412.

Simpson, J. R. and W. C. Evans. 1953. "The Metabolism of Nitrophenols by Certain Bacteria." Biochem. J. 55: xxiv.

Sollmann, Torald. 1949. "Correlation of the Aquarium Goldfish Toxicities of Some Phenols, Quinones and Other Benzene Derivatives with their Inhibition of Autooxidative Reactions." J. General Physiol. 32: 671-679.

Stanier, R. Y. 1950. "Problems of Bacterial Oxidative Metabolism." Bact. Rev. 14: 179.

Stanier, R. Y., M. Doudoroff, and E. A. Adelburg. 1970. The Microbial World, 3rd. ed. Prentice-Hall, Inc., Englewood Cliffs, NJ.

Stringer, G. E. and R. G. McMynn. 1958. "Experiments with Toxaphene as a Fish Poison." Can. Fish Culture 23: 39-47.

Summerfelt, R. C. and W. M. Lewis. 1967. "Repulsion of Green Sunfish by Certain Chemicals." J. WPCF 39: 2030-2038.

Symons, J. M. and McKinney, R. E. 1958. "The Biochemistry of Nitrogen in the Synthesis of Activated Sludge." Sewage and Ind. Wastes 30: 875.

Tarkowski, S. 1968. "Effect of Acrylonitrile on Certain Properties of Cytochrome Oxidase." Med. Prac. 19: 525-531.

Tarzwell, C. M. 1959. "The Toxicity of Some Organic Insecticides to Fishes." Proc. 12th Annual Conf., Southeast Assoc. Game and Fish Comm. pp. 233-239.

Taylor, G. I. 1954. "The Dispersion of Matter in Turbulent Flow through a Pipe." Proc. Royal Soc. of London 223: 446.

Toenniessen, G. H. and R. A. Mah. 1971. "The Methane Fermentation of Acetate and Methanol." Bacteriol. Proc. 71: 17.

Trama, F. B. 1955. "The Acute Toxicity of Phenol to the Common Bluegill." Notulae Natur. 269: 1-10.

Turnbull, H., J. DeMann, and R. Weston. 1954. "Toxicity of Various Refinery Materials to Freshwater Fish." Ind. Eng. Chem. 46: 324-333.

Ukeles, R. 1962. "Growth of Pure Cultures of Marine Phytoplankton in the Presence of Toxicants." App. Microbiol. 10: 532-537.

Unz, R. F. and S. R. Farrah. 1972. "Use of Aromatic Compounds for Growth and Isolation of Zooglea." Appl. Microbiol. 23: 524-530.

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Wallen, I. E., W. C. Greer, and R. Lasater. 1957. "Toxicity to *Gambusia affinis* of Certain Pure Chemicals in Turbid Waters." Sewage and Ind. Wastes *29*: 695-711.

Warnick, D. C. 1966. "Growth Rates of Yellow Perch in Two North Dakota Lakes After Population Reduction with Toxaphene." U. S. Fish and Wildlife Service, Bureau Sport Fisheries and Wildlife Resource Publ. 9.

Weiss, C. M. 1964. "Organic Pesticides and Water Pollution." Public Works *95*: 84-87.

Welch. H. and G. G. Slocum. 1943. "Relation of Length of Carbon Chain to the Primary and Functional Toxicities of Alcohols." J. Lab. and Chem. Med. *28*: 1440.

Wilkinson, J. F. 1958. "The Extra Cellular Polysaccharides of Bacteria." Bact. Rev. *22*: 46.

Wollitz, R. E. 1963. "Effects of Certain Commercial Fish Toxicants on the Limnology of Three Cold-Water Ponds, Montana." Proc. Montana Acad. of Sci. *22*: 54-81.

Woodward, H. F., Jr. 1967. "Methanol." In: *Kirk-Othmer Encyclopedia of Chemical Technology*. Interscience Publishers, NY *13*: 370-398.

Workman, G. W. and J. M. Neuhold. 1963. "Lethal Concentrations of Toxaphene for Goldfish, Mosquito Fish, and Rainbow Trout, with Notes on Detox-ification." Progressive Fish Culture *25*: 23-30.

Wuhrmann, K. 1955. "Factors Affecting Efficiency and Solids Production in the Activated Sludge Process." Proc. of Conf. on Biol. Waste Treatment, Manhattan College, NY.

Zanoni, A. E. 1969. "Secondary Effluent Deoxygenation at Different Temperatures." J. WPCF *41*: 640.

APPENDIX A

HYPOTHESIS TESTS

A-1. INFERENCE ABOUT MICHAELIS-MENTEN CONSTANT, k_s, FOR PHENOL.

$$H_o: K_{s(28\ ^oC)} = \overline{K}_s$$

$$H_1: K_{s(28\ ^oC)} \neq K_s$$

$$\overline{K}_s = 236 \text{ mg/l}$$

$$K_{s(28\ ^oC)} = 206.3 \text{ mg/l}$$

$$S_{K_{s(28\ ^oC)}} = 55.6 \text{ mg/l}$$

$$n = 29$$

Reject H_o if $|T.S.| \geq t_{n-2,0.99}$

$$T.S. = \frac{206.3 - 236}{55.6} = 0.534$$

$$t_{27,0.99} = 2.4727$$

$$|T.S.| < t_{27,0.99}$$

H_o is not rejected. Therefore, the mean, $K_s = 236$ mg/l, can be represented as the Michaelis-Menten constant for all test temperatures.

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A2. INFERENCE ABOUT CELL YIELD COEFFICIENT, a, FOR PHENOL.

$H_o : a_{(28\ ^oC)} = \bar{a}$

$H_1 : a_{(28\ ^oC)} = \bar{a}$

$a = 1.21$

$a_{(28\ ^oC)} = 1.048$

$S_{a(28\ ^oC)} = 0.069$

$n = 29$

Reject H_o if $|T.S.| \geq t_{n-2,0.99}$

$T.S. = \frac{1.048 - 1.21}{0.069} = -2.348$

$t_{27,0.99} = 2.4727$

$|T.S.| < t_{27,0.99}$

H_o is not rejected. Therefore, the mean, a = 1.21, can be represented as the cell yield coefficient for all test temperatures.

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A-3. INFERENCE ABOUT a' AND b' FOR PHENOL.

$$H_0 : \overline{Rr_1 - Rr_2} = 0$$
$$H_1 : \overline{Rr_1 - Rr_2} \neq 0$$

$$\overline{Rr_1 - Rr_2} = 0.73$$

$$S_{(\overline{Rr_1 - Rr_2})} = 5.68$$

$$n = 10$$

Reject H_0 if $|T_9| \geq t_{9,0.75}$

$$T_9 = \frac{0.73}{5.68/\sqrt{10}} = 0.406$$

$$t_{9,0.75} = 0.7027$$

$$|T_9| < t_{9,0.75}$$

H_0 is not rejected. Therefore, there is no evidence that the estimated oxygen uptake rates based on a' = 1.39 and b' = $1.42k_d$ are different from the actually observed rates.

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A-4. INFERENCE ABOUT a' AND b' FOR METHANOL.

$$H_0 : \overline{Rr_1 - Rr_2} = 0$$
$$H_1 : \overline{Rr_1 - Rr_2} \neq 0$$

$$\overline{Rr_1 - Rr_2} = 0.317$$

$$S_{(\overline{Rr_1 - Rr_2})} = 13.0$$

$$n = 81$$

Reject H_0 if $|T_{80}| \geq t_{80,0.75}$

$$T_{80} = \frac{0.317}{13/\sqrt{81}} = 0.219$$

$$t_{80,0.75} = 0.6776$$

$$|T_{80}| < t_{80,0.75}$$

H_0 is not rejected. Therefore, a' = 2.23 and b' = 1.42 k_d can be used to estimate the oxygen uptake rate in methanol decomposition.

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APPENDIX B

METHANOL STRIPPING

The boiling point of methanol is 64.7° C at 1 atm, thus it is highly volatile. As pointed out previously, considerable amounts of methanol were removed by volatilization; therefore, methanol stripping as well as biological decomposition, can play an important role in methanol removal.

Eckenfelder et al. (1957) formulated the stripping kinetic model as:

$$\frac{dS}{dt} = -k_v\left(\frac{Q_a}{V}\right)^c S, \qquad \text{(B-1)}$$

where:
k_v = volatilization rate coefficient,
Q_a = stripping air flow rate (L^3/T),
c = a constant, and
V = reactor volume (L^3).

However, there was a disagreement regarding the value of the constant, c (Engelbrecht et al., 1961; Gaudy et al., 1961). Eckenfelder et al. (1957) reported that the c value ranged from 0.15 to 0.35, while Engelbrecht et al. (1961) reported that c was about 0.85 rather than 0.35. Since Equation B1 has no rational basis, the following air stripping kinetics theory was developed.

The widely accepted gas transfer equation is:

$$V\frac{dS}{dt} = -k_v A_a(S - S_s), \qquad \text{(B-2)}$$

where:
V = reactor volume (L^3),
k_v = volatilization rate coefficient (L/T),
A_a = air-water interface area (L^2), and
S_s = saturated volatile substance concentration for a given partial pressure (M/L^3).

The vapor pressure of methanol in atmosphere can be regarded as zero. The rate coefficient, k_e, in Equation 59 is equivalent to $k_v \frac{A_a}{V}$ in Equation

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B-2. When 1 liter air/min/liter water was supplied in the methanol removal study, the maximum value of k_e was 0.0277 hr^{-1} at 28°C. This means that the saturated methanol concentration (S_s) for the methanol vapor pressure in the stripping air bubble also is negligible*. Equation B-2 can be reduced to:

$$\frac{dS}{dt} = -k_v \frac{A_a}{V} S \qquad \text{(B-3)}$$

When air is supplied with an average bubble size of D_a (diameter) at an air flow rate of Q_a, the air-water interface area is solved to be:

$$A_a = e A_s + \frac{6 Q_a t_d}{D_a} \qquad \text{(B-4)}$$

where: e = surface area expansion coefficient caused by turbulence,
A_s = surface area of water body at quiescent condition (L^2),
Q_a = air flow rate (L^3/T),
D_a = average diameter of air bubble (L),
t_d = detention time of air bubble in the reactor (H/v_b),
H = depth of reactor (L), and
v_b = bouyancy velocity of air bubble (L/T), a function of D_a.

Therefore, Equation B-3 becomes:

$$\frac{dS}{dt} = -k_v \left(\frac{e}{H} + \frac{6 Q_a H}{D_a v_b V}\right) S. \qquad \text{(B-5)}$$

As shown in Equation B-5, the stripping rate largely depends on the geometry of the reactor tank, air bubble size, and the air flow rate. Equation B-5 is graphically interpreted in Figure B-1. The reactor depth that provides the minimum stripping rate, H_{min}, is solved by setting Equation B-5=0, or:

$$H_{min} = -\sqrt{\frac{e D_a v_b V}{6 Q_a}} \qquad \text{(B-6)}$$

The stripping rate increases as the reactor depth increases or decreases from H_{min}. As the reactor depth decreases from H_{min}, volatilization through the surface area of the water body dominates and as the reactor depth increases from H_{min}, air stripping by air bubbles dominates.

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* The saturated methanol concentration was calculated based on the data from Lang's Handbook of Chemistry (Dean, 1973).

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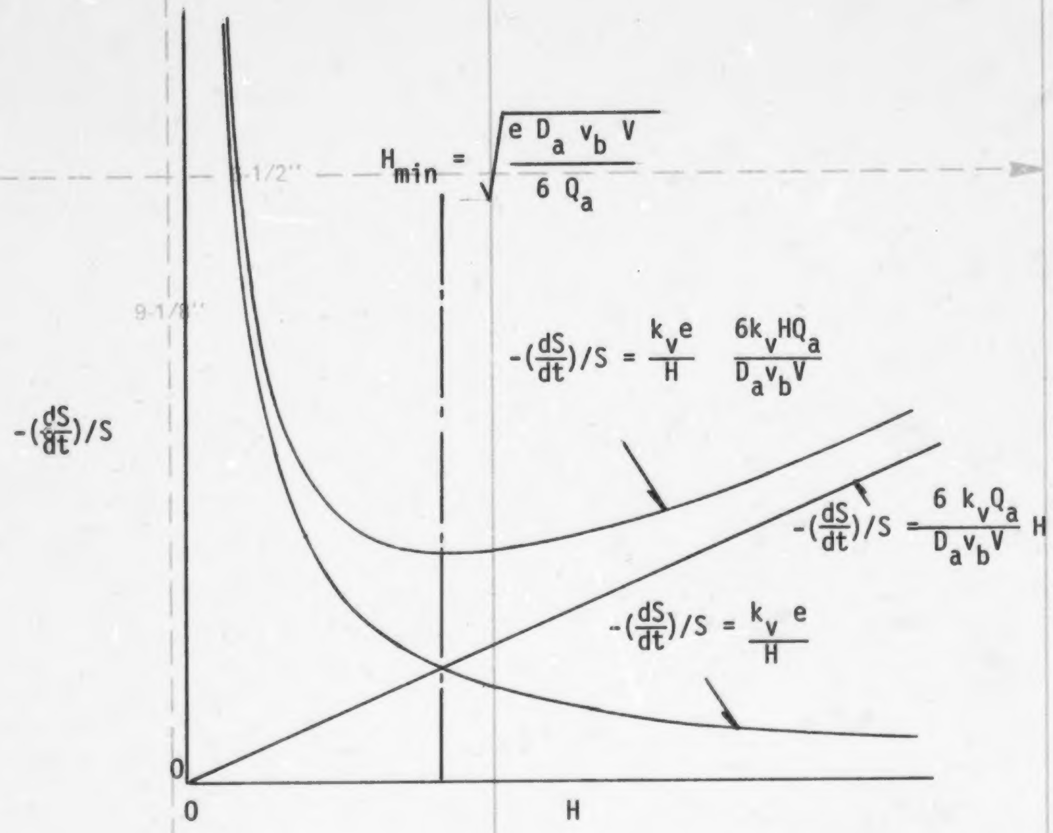

Figure B-1. The relationship between the air stripping rate and the reactor depth.

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At a quiescent condition ($A_a = A_s$), the volatilization rate coefficient, k_v, was estimated to be 0.07116 cm/hr at 5^{0} C, 0.2044 cm/hr at 22^{0} C, and 0.5973 cm/hr at 28^{0} C (Figure B-2). As temperature neared the boiling point, the volatilization rate coefficient sharply increased, so that the Arrhenius equation failed to describe the temperature effect on the rate coefficient. When 1 liter air/min/liter water of air was supplied through glass tubes with a diameter 0.3 cm into the 3-liter reactors with a surface area (A_s) of 105 cm^2, the air-water interface area increased by 32.5% ($A_a = 1.325\ A_s$).

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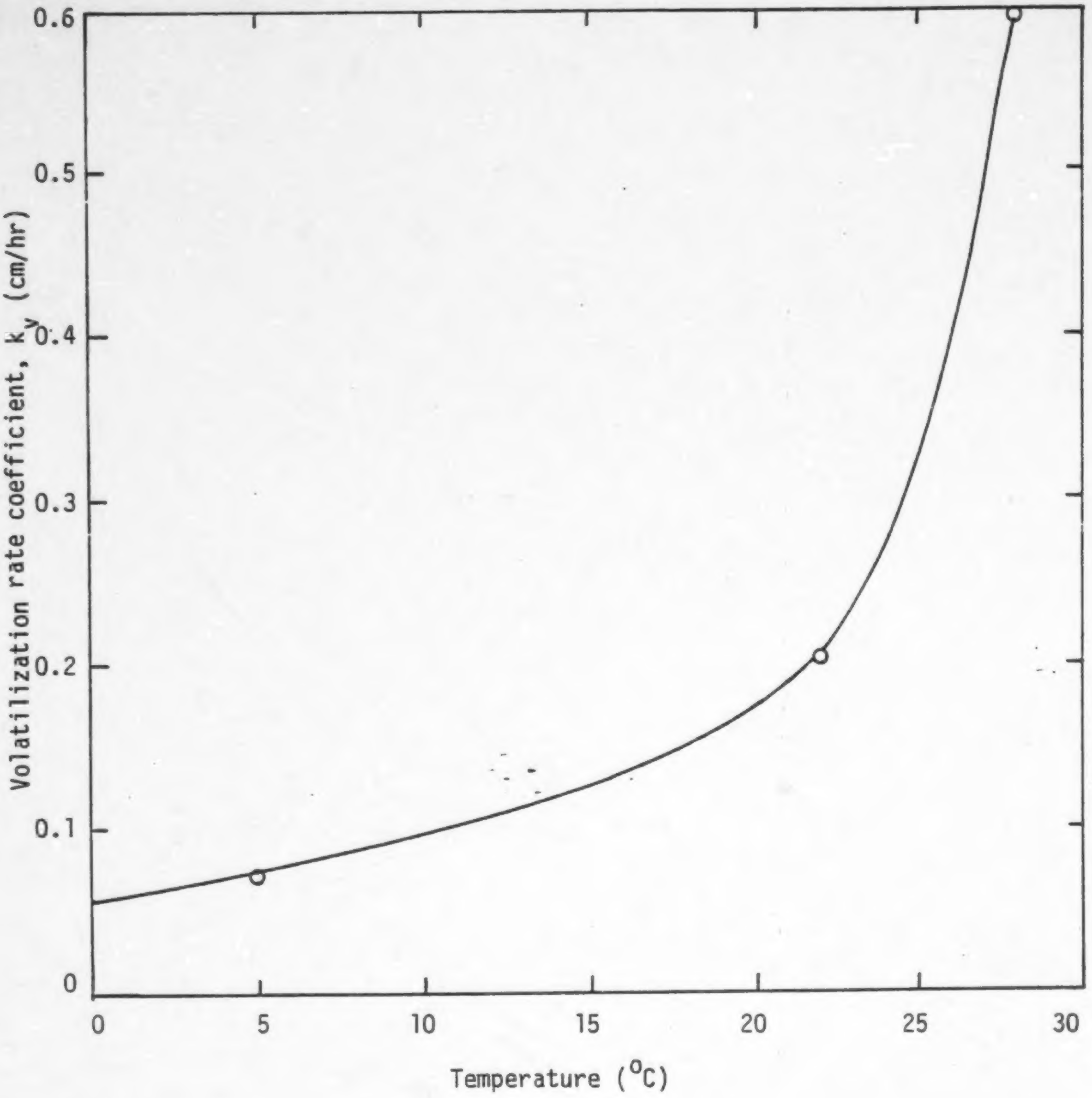

Figure B - 2. Temperature effect on the volatilization rate coefficient.

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APPENDIX C

TABLE C-1 COMPUTATION RESULTS OF CLOTH BAG EFFICIENCY, BACTERIAL GROWTH, AND AERATION TIME FOR THE CLOTH BAG APPLICATION TESTS IN BATCH REACTOR (REACTOR #1)

S (mg/l TOC)	E	Ek (hr^{-1})	X_c (mg/l VSS)	X (mg/l VSS)	t (hrs.)
660			6,780	380.0	0
650	0.7932	0.2221	6,905	387.0	0.327
640	0.7890	0.2209	7,031	394.0	0.656
630	0.7848	0.2198	7,157	401.1	0.987
620	0.7806	0.2186	7.284	408.2	1.320
610	0.7764	0.2174	7,411	415.4	1.655
600	0.7722	0.2162	7,538	422.5	1.992
590	0.7679	0.2150	7,667	429.7	2.332
580	0.7637	0.2138	7,795	436.9	2.674
570	0.7594	0.2126	7,924	444.1	3.019
560	0.7551	0.2114	8,053	451.4	3.368
550	0.7508	0.2102	8,183	458.6	3.719
540	0.7465	0.2090	8,313	465.9	4.074
530	0.7422	0.2078	8,443	473.2	4.432
520	0.7379	0.2066	8,574	480.5	4.793
510	0.7336	0.2054	8,705	487.9	5.159
500	0.7293	0.2042	8,838	495.2	5.529
490	0.7249	0.2030	8,967	502.6	5.903
480	0.7206	0.2018	9,098	509.9	6.282
470	0.7163	0.2006	9,229	517.3	6.666
460	0.7120	0.1994	9,361	524.7	7.055
450	0.7077	0.1981	9,492	532.0	7.449
440	0.7034	0.1969	9,624	539.4	7.850
430	0.6991	0.1957	9,755	546.8	8.256
420	0.6948	0.1945	9,887	554.1	8.669
410	0.6905	0.1933	10,018	561.5	9.089
400	0.6862	0.1921	10,149	568.8	9,516
390	0.6820	0.1910	10,280	576.2	9.950
380	0.6777	0.1898	10,411	583.5	10,393
370	0.6735	0.1886	10,541	590.8	10,845
360	0.6692	0.1874	10,671	598.1	11.306
350	0.6650	0.1862	10,801	605.4	11.776
340	0.6608	0.1850	10,930	612.6	12.257

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TABLE C-1 (Continued)

S (mg/1 TOC)	E	Ek (hr^{-1})	X_c (mg/1 VSS)	X (mg/1 VSS)	t (hrs.)
330	0.6567	0.1839	11,059	619.8	12.750
320	0.6525	0.1827	11,187	627.0	13.254
310	0.6484	0.1816	11,314	634.1	13.771
300	0.6443	0.1804	11,441	641.2	14.302
290	0.6402	0.1793	11,567	648.3	14.847
280	0.6361	0.1781	11,692	655.3	15.409
270	0.6321	0.1770	11,816	662.2	15.987
260	0.6281	0.1759	11,938	669.1	16.584
250	0.6241	0.1748	12,060	675.9	17.201
240	0.6202	0.1737	12,180	682.6	17.839
230	0.6163	0.1726	12,299	689.3	18.502
220	0.6125	0.1715	12,416	695.9	19.190
210	0.6087	0.1704	12,530	702.3	19.906
200	0.6049	0.1694	12,643	708.6	20.654
190	0.6012	0.1683	12,754	714.8	21.436
180	0.5975	0.1673	12,862	720.9	22.257
170	0.5939	0.1663	12,966	726.7	23.121
160	0.5904	0.1653	13,068	732.4	24.034
150	0.5869	0.1643	13,165	737.9	25.001
140	0.5835	0.1634	13,258	743.1	26.031
130	0.5802	0.1625	13,346	748.0	27.133
120	0.5770	0.1616	13,427	752.6	28.319
110	0.5739	0.1607	13,502	756.8	29.604
100	0.5710	0.1599	13,568	760.5	31.008
90	0.5681	0.1591	13,624	763.6	32.555
80	0.5656	0.1584	13,667	766.0	34.281
70	0.5632	0.1577	13,694	767.5	36.233
60	0.5611	0.1571	13,699	767.8	38.485
50	0.5594	0.1566	13,676	766.5	41.147
40	0.5582	0.1563	13,612	762.9	44.408

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APPENDIX D

APPLICATION OF CLOTH BAGS IN ONE-DIMENSIONAL DISPERSION SYSTEM

When the dispersion coefficient for cloth bags is different from the dissolved pollutant dispersion coefficient, the total mass of the pollutant at time t is solved to be:

$$\frac{M_s}{M_{so}} = \exp\left\{-\frac{EkM_x}{\sqrt{\pi(D+D')}\quad ArK_s}\left(\sqrt{t}-\sqrt{t_a}\right)-k_r t\right\}$$

where: D' = dispersion coefficient for cloth bags.

It appeared from a visual dispersion study of twenty cloth bags in the model river that D' was not significantly different from D. However, if D' = ½D, then:

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$$\frac{M_s}{M_{so}} = Y^{1.15}\, e^{-k_r t},$$

where:

$$Y = \exp\left\{-\frac{EkM_x}{\sqrt{2\pi D}\,ArX_s}\left(\sqrt{t}-\sqrt{t_a}\right)\right\}$$

Therefore, the difference in D and D' does not significantly affect the removal rate.

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